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INFO FILE VR OCA/SOV3, CGS/HSG, OCA/CSS

CR/4, OPR4, DER, [redacted] N10/USSR,

SE7, SS3, PS/EG2, OCD3, OSI/LSD

T 508716 EIA288 PAGE 01-01 NC 40942

STATSPEC

TOR:032335Z DEC 75

file

P 031242Z DEC 75
FM AMEMBASSY MOSCOW
TO SECSTATE WASHDC PRIORITY 7391
BT
UNCLAS MOSCOW 17318
E.O. 11652: NA
TAGS: TGEN, US, UR
SUBJ: US-USSR COOPERATION IN S&T: MICROBIOLOGY: MICROBIOLOGICAL CONTROL OF PESTS OF AGRICULTUREAL CROPS (01.07-05)
REF: A) STATE 280402, B) MOSCOW 16760
1. EMBOFF 3 DECEMBER EXPLAINED TO KOTOV US-SIDE DESIRE TO HOST SOVIET GROUP BUT NEED FOR MORE TIME FOR PLANNING. KOTOV REPLIED THAT SOVIETS THOUGHT THIS WAS THE CASE, SINCE THEY HAD NOT YET RECEIVED VISAS.
2. PER REF A, EMBOFF PROVIDED SCHEDULE PROPOSAL OF 18 JANUARY - 1 FEBRUARY AND LIST OF SITES FOR VISIT. KOTOV FEELS THAT BOTH SCHEDULE AND LIST OF SITES WILL PLEASE SOVIET DELEGATION.
3. EMBOFF HAS SENT LETTER (REQUESTING CONFIRMATION SOONEST OF ACCEPTABILITY) TO KOTOV, COPY TO DVORETS. STOESSEL

01.07-54T Microbiology

LFSS

State Dept. declassification & release instructions on file

USSR Recycles Animal Waste to Produce Yeast Single Cell Protein: The USSR is using swine waste as a cultural base for growing yeast single cell protein (SCP) at the Telenskiy swine fattening complex, north of Kishinev. The daily production of dried yeast is 1.5 tons from a facility which has a total capacity of 10,000 hogs and a turnover rate of 2.2 times per year. The SCP process involves hydrolysis, separation, and fermentation of the raw waste. [redacted]

25X1C

Comment: This is the first indication that the Soviets are recycling animal waste in the production of yeast SCP. The most likely application for this product is its use as a feed supplement for animals. Although this facility represents a minor production effort, it demonstrates the dual benefits of reducing waste disposal problems and providing a high protein feed supplement for swine.

Assuming that shoats weighing about 40 pounds were fattened to a market weight of 200 pounds, approximately 1,760 tons live-weight of swine would be produced at the Telenskiy complex, annually. If a nominal feed conversion ratio of 5 to 1 were realized, approximately 8,800 tons of feed would be consumed. The addition of 6 percent yeast SCP to the feed instead of soybean meal and fish meal would save about 530 tons of these high protein feed stuffs at the Telenskiy operation alone. [redacted]

25X1A

25X1C

Agricultural Technology Receives Priority in Soviet Five-Year Plan: USSR announced that expenditures on the agricultural sector would be increased nearly a third over the next 5 years. The Soviets expect to further strengthen the resource and technical base of agriculture and to improve the organization of agricultural production. Planners expect a more efficient use of arable land, machinery and equipment and other resources. They also will insist on improved quality of agricultural products. Increased mechanization and production are expected to increase farm labor productivity by 13 percent above the 1971-75 level. Additional large-scale complexes for raising livestock, processing agricultural commodities and grain storage facilities are to be constructed.

S&T — Microbiology

Plans call for additional irrigation and drainage for improving range lands and for continuing the program to develop agriculture in the Non-Black Soil Zone in the RSFSR. Supplies of fertilizer and feed additives are to be increased. (FBIS-SOV-75-238, 10 Dec 75) (U)

Comment: The plan indicates a continuation with no major changes in direction of the Soviet effort to update agricultural science and technology. Improvements in Soviet agrotechnology probably will be gradual but slow, despite recent large purchases of equipment, licenses, and other R&D items from the US and other western countries.

Apart from technology, weather will continue to play a major role in determining the level of USSR farm output. Impediments to USSR agriculture will continue to be its climatic-geographic restrictions, insufficient autonomy and incentives for farm managers and workers, and inefficient administration of agricultural research and development institutions. [redacted] 25X1A 25X1C [redacted]

Libya Seeks Hydroponic Project for Vegetable Production: The Libyan Government has recently indicated that it is prepared to sign a contract with a US firm for the purchase of a number of hydroponic greenhouse units to increase Libyan food production. The contract calls for a three-phase program over a 4-year period. At the end of that time, the country would have 32 operating units covering more than 30 acres, 250 trained personnel, and an annual production of approximately 5,000 tons of vegetables selected from a list of 14. If this project, near the village of Gharyan, is successful, similar projects will be started at three other locations. The total Libyan budget for hydroponic units is $100 million. (C)

Comment: This investment in hydroponics (growth of plants in nutrient solutions) might prove to be worthwhile. It offers the Libyans a new technology and will give them a much-needed additional vegetable production capability. Hydroponics has been tried in a number of places and, although yields have been high under care-

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INFO RF. FILE VR. DDS 3, D&D3, CR/4, LSN/AAO, CCS/HSC, SE 7, OSI, DDST-2

T 485935 EIA021 PAGE 01-01 NC 32883

TOR:242106Z NOV 75

R 241430Z NOV 75
FM AMEMBASSY MOSCOW
TO RUEHC/SECSTATE WASHDC 7065
RUEHFB/DIRECTOR FBI
BT
UNCLAS MOSCOW 16876
E.O. 11652: N/A
TAGS: CVIS UR (SKLADNEV, ANATOLIY ALEKSANDROVICH)
SUBJECT: VISAS SPLEX: SCIENCE AND TECHNOLOGY AGREEMENT:
THE MICROBIOLOGICAL CONTROL OF PESTS OF AGRICULTURAL CROPS
01.07.05
REF: (A) MOSCOW 16367; (B) MOSCOW 16760
FOR SCA/VO AND OES/SCI
VISAS DONKEY CHIPMUNK

file (S+T) Microbiology

1. SKLADNEV, ANATOLIY ALEKSANDROVICH
   25 AUG 1925 GORLOVKA
   CHIEF OF TECHNOLOGICAL ADMINISTRATION, CENTRAL ADMINISTRATION
   OF MICROBIOLOGICAL INDUSTRY, USSR COUNCIL OF MINISTERS
2. ETD DEC 5, STAY 14 DAYS.
3. REFTEL B PROPOSED SIX MEMBER SOVIET DELEGATION, BUT SKLADNEV
IS GOING IN PLACE OF I.K. CHEREMUKHIN, SO THE DELEGATION WILL
STILL CONSIST OF 6 MEMBERS. MATLOCK

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INFO RF. FILE. VR OES/SOV3, N/USSR, OPR 4, OER 4, SRA, [redacted], OES/LSD, S/S-S 2, SE 7, SCA 3,

STATE MESSAGE

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3		6	

T 478892 EIA134 PAGE 01-01 NC 30373

STATSPEC

TOR: 210846Z NOV 75

File - Microbiology (OES/T Subject)

P 210826Z NOV 75
FM AMEMBASSY MOSCOW
TO SECSTATE WASHDC PRIORITY 6996
BT
UNCLAS MOSCOW 16760
DEPT PLEASE PASS NSF FOR LEISE; USDA FOR EDMINSTER, ARS
E.O. 11652: N/A
TAGS: TGEN TBIO US, UR
SUBJECT: US-USSR COOPERATION IN S&T: MICROBIOLOGY: SECTION 5:
MICROBIOLOGICAL CONTROL OF INSECT PESTS OF AGRICULTURAL CROPS
(01.07.05)
1. EMBASSY RECEIVED 21 NOVEMBER UNDATED LETTER FROM V. SEREGIN
TO J.M. LEISE, NATIONAL SCIENCE FOUNDATION.
2. LETTER ANNOUNCES THAT SIX-PERSON SOVIET DELEGATION UNDER SUBJECT
AREA PLANS TO TRAVEL TO THE US 5 DECEMBER.
3. SOVDEL WOULD LIKE TO VISIT THE RESEARCH CENTER AT
BELTSVILLE, BROWNSVILLE LAB, ENTOMOLOGY DEPARTMENT OF THE UNI-
VERSITY OF CALIFORNIA, THE RESEARCH CENTER OF THE FIRM
"INTERNATIONAL MINERAL AND CHEMICAL CORPORATION," THE LABOR-
ATORY OF DR. ROGOV, AND ABBOTT LABS.
4. LETTER REQUESTS CONFIRMATION OF AGREEMENT TO RECEIVE SOVDEL.
5. LETTER POUCHED REGISTRY NUMBER 1907939
MATLOCK

01.07-S&T Microbiology

[redacted]

AS

STATINTL

SUBJ: MICROBIOLOGY: MOLECULAR BIOLOGY (01.0703)

US/USSR SEMINAR ON THE GENETICS OF YEAST AND ENTOMOPATHOGENIC MICROORGANISMS

Leningrad, USSR

November 17 - 21, 1975

Harlyn O. Halvorson
Brandeis University
Project Coordinator

FILE 01.07 Microbiology

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22 MAR 1976

Approved For Release 2001/08/27 : CIA-RDP79-00798A000400110001-1

Table of Contents

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1. Background of the conference

At the very onset of the US/USSR program in microbiology, Drs. Mortimer, von Borstel and myself attended a national USSR conference in Armenia at which time we were exposed to the research efforts on yeast genetics in the Leningrad area. In particular, their claim to have mutagenized Saccharomyces cerevisiae to convert it into a Candida type yeast drew our attention and our interest in an early meeting with the Leningrad group. This meeting was built into the exchange program and coupled with the need to reduce the total number of meetings led to a combination meeting involving the genetics of yeast and entomopathogenic microorganisms. This conference was confirmed in a meeting last September in Moscow attended on my behalf by Dr. Robert Mortimer (USA) and by Dr. Sukhodolets (USSR). Because the major portion of the conference in Leningrad would deal with yeast genetics, details of the planning were carried out between Dr. Inge-Vechtomov (USSR) and H. O. Halvorson (USA).

The USA delegation was selected to reflect these two interests. With regard to studies on yeast, individuals were selected knowledgeable in the techniques, methodology and principle regarding mutation, genetic organization, recombination, the mating system, and the genetic manipulation of yeast for hydrocarbon utilization and the production of amino acids. For entomopathogenic microorganisms, we selected experts active in research on Bacillus thuringiensis. Dr. Thorne, who has carried out the only extensive genetic analysis of this organism in the United states, and Dr. Yousten who has long been interested in the physiological studies on B.thuringiensis and active in attempting to derive a genetic exchange system in this organism.

In addition to the seminar itself we requested the opportunity of visiting the Department of Genetics of Leningrad University (a department of historical significance in the early development of genetics, particularly in the 1920s) and the laboratory of radiation genetics of the Leningrad Institute of Nuclear Physics.

To prepare for the meeting the American delegation left a day early and stopped over in Stockholm for both a rest and a briefing for the upcoming conference.

2. Overall program for the Seminar.

The American delegation landed at the Pulkovo Airport in Leningrad where we were met by our Russian hosts. We were brought by bus to the Hotel Sovetskaja, a recently built hotel for conferences in the Leningrad area. After checking into the hotel we were taken to Demjamova Uha, a restaurant in Leningrad where opening toasts and exchanges took place regarding the upcoming meeting.

The conference itself was held in the House of Scientists, a residence of a former aristocrat in Russia which was donated (!) for a conference site. It is an old but extremely well built house filled with art objects, conservatory, dining room, etc., well suited for this purpose. The scientific sessions started around 10:00 and would continue to about 2:30 at which time dinner was served in the House of Scientists. Following dinner discussion continued to about 5:30 at which time we returned to our hotel. On Monday evening, November 17, we were taken to the ballet Jiesel, on Tuesday afternoon we were given a rapid tour of the Hermitage which is almost adjacent to the conference site and on that evening entertained at the opera Prince Igor. Wednesday afternoon involved a visit to the Department of Genetics and Breeding in Leningrad University and in the evening a visit to the circus. Friday afternoon the group visited the laboratory of radiation genetics of the Leningrad Institute of Nuclear Physics, supplemented later by a shopping expedition and visits to Leningrad. Ample opportunity was given both during the morning and afternoon sessions for discussions, both in large and small groups of areas of mutual interest.

Individual sessions were jointly chaired by a US and a USSR participant. Two translators, both scientists active in related research areas, were available throughout the meeting in addition to which many of the Russians were capable of assisting in translation. Many of the young scientists spoke sufficient English to make it possible for us to carry on discussions with them informally, both during and after the formal meeting sessions. We were fortunate to have our scientific hosts as our guides in visiting Leningrad, so that our visits were relaxed and informal, constantly interspersed with scientific discussions.

Mapping of Mutations in the ade2 locus in Saccharomyces cerevisiae, V. V. Kuasha, D. A. Gorodenin, S. G. Inge-Vechtomov (Zhdanov Leningrad State University). Attempts were made to order mutational sites in ade2 gene by recombination rates of two, three and four-point corsses. Suppressible ade2 mutants were coupled with a suppressor and new adenine-red mutants were isolated with the suppressed strain. Some of the new adenine-requirements were due to second mutations within the ade2. By crossing out the suppressor, one could recover strains with double point-mutations. These double point-mutants were crossed to a series of single point-mutants as well as other double point-mutants. Both UV-induced recombination rates and spontaneous recombination rates were determined with diploid strains consisting of two-point, three-point and four-point crosses. The spontaneous rates were determined by the Luria-Delbruck fluctuation test, using a new procedure of spotting diluted suspensions on the surface of thick-agar plates that contained limited amounts of adenine. Apparently there was a consistent level of residual growth over each area of the inocula. Recombinants produced colonies growing over the background growth, and the recombination rates were calculated from the number of spotted areas that did not contain any recombinants. If recombination occurred in a three-point cross either spontaneously or after UV-induction, it was assumed that the site of the single mutant fell outside the region encompassed by the double mutant. Similar considerations were given for ordering with four-point corsses. A fine-structure map was constructed from these results. The direction of translation of the gene was infered from the fine-structure map and from complementing nonsense

mutants and polar complemention mutants. From the discussion that followed the presentation, it was generally accepted that the order of mutational sites should be considered only approximate and not definitive.

Interallelic Complementation in ade2 Locus and its Variability, T. R. Sodidla, N. P. Mikhailova (Zhdanov Leningrad State University). An extensive complementation map was constructed from a large number of crosses of ade2 mutants. From the results of their and other works, the authors have defined the smallest segment of complementation as a "veron". Mutants belonging to the same veron do not complement each other; mutants of one veron will complete some or all mutants of other verons. The ade2 complementation map is composed of 12 verons, I through XII. The I through VIII verons can be considered as a linear array, with complementing pairs of mutants always represented as overlapping continuous lines and complementing pairs as nonoverlapping lines. The verons IX through XII could not be represented as a continuous line on a linear map. Instead of representing the map in the customary way as a complicated geometrical figure, the results were presented by broken lines with discontinuous regions of complementation. It was suggested that the verons were related to structure of the enzyme. Mutants having common properties were often found to belong to the same veron. For example 88% of the mutants that were stimulated by CO_2 belonged to veron VIII. It should be mentioned that such consideration are not new and that such approach for analyzing mutant characteristics previously have not proved to be fruitful.

Mutagenic Effect of 6-Hydroxyl-aminopurine in Yeast S. cerevisiae, B. V. Simarov, A. Shanki, N. N. Khromov-Borisov (Zhdano Leningrad State University). Evidence was presented that 6-hydroxyl-aminopurine (HAP) is highly mutagenic in yeast. HAP was used to induce anxto-trophic mutants, especially adel and ade2 mutants. This is the first base analogue that has been shown to be highly mutagenic in yeast. However the action of HAP is unclear. HAP will inefficiently substitute for adenine in the growth requirement of ade2 mutants, indicating that it may act as an adenine analogue. However, mutagenic treatments are performed in the presence of yeast extract which should contain relatively high levels of adenine and which should counteract the action of HAP.

A systematic study of the frequencies of adel and ade2 mutants induced by HAP and a variety of other mutagens was presented and the following is a summary of some of the results:

Mutagen	Ratio ade2/adel	Rank
X-rays	1.7	1
ICR	2.1	2
UV	2.5	3
EMS	4.3	4
HND_2	9.0	5
HAP	60.7	6

The same rank, excluding HAP, was previously observed for ratios of ade6 and ade7 mutants in Schizosaccharomyces pombe and of ade3A and ade3B in Neurospora crassa. It was concluded that the rank of a mutagen is a reflection of its specificity, such that HAP would be considered highly specific while X-rays would be considered to have little specificity. The meaning and interpretation of "mutagen specificity" was discussed after the presentation. In the present context, "mutagen specificity" indicates the distinctive action on two different genes and does not necessarily imply a selective type of nucleotide change.

Studies on the Mechanism of Recessive Super Suppressors, V. N. Smirnov

(Institute of Cardiology of the USSR Academy of Sciences), L. V. Lyzlova, S. G. Inge-Vechtomov (Zhdanov Leningrad State University). Perhaps one of the most interesting papers was concerned with the mechanism of action of recessive nonsense-suppressors which were temperature sensitive for growth. A large portion of the presentation can be found in the publication: Recessive super-suppression in yeast by V. N. Smirov, V. G. Kreier, L. V. Lyzlova V. M. Andrianov, and S. G. Inge-Vechtomov. Mol. Gen. Genetics, 129, 105-121 (1974). It was demonstrated that these recessive suppressors carry a defect in protein synthesis leading to temperature sensitivity of translation. Examination of separate translational events in the normal strain and mutant strains indicated that recessive suppression is most likely caused by a mutant protein participating in or affecting the termination of peptide chains on ribosomes. Evidence that indicated the ribosomes were altered in the suppressor strains came from the differences of dissociation of the 80s ribosome in 0.3M KCI and the different concentration of Mg^{++} for optimum protein synthesis. It appeared as if the mutations altered the ribosome, causing reading of nonsense codons. Thus these recessive suppressors are distinct from the dominant suppressors which act by having mutated tRNAs that read nonsense codons.

I. A. Zaharov - Cytoduction in S. cerevisiae.

In a small fraction of matings (1-5%) two cells will fuse and form a dikaryon; however, before karyogamy (nuclear fusion) a zygate may produce a bud containing a haploid nucleus but in a mixed cytoplasm. For example in the mating a $\rho+$ pet^- x $\alpha\rho^-$ ade 1 (red) most colonies will be phenotypically non-mating, $\rho+$, and white (wild type for adenine). Rare cytoductants ($\alpha\rho+$ ade 1) are formed. In one strain, cytoduction constitutes 40% of the matings. A propensity for cytoduction is dominant. Both Xrays and UV light increase cytoduction. The UV-sensitive mutation rad 54 leads to nearly 100% of all matings when the parents are irradiated before mating.

In the discussion following the paper, Dr. G. R. Fink reported the isolation of a recessive cytoduction mutation (cyt 1) which is apparently centromere linked and which fails to give normal karyogamy in 99% of all matings.

G. I. Naumov - Genes controlling homothallism in yeast.

A brief review of genes controlling homothallism was presented. The scheme was devised after extensive genetic analysis of crosses of homothallic and heterothallic strains. Five genes controlling conversion of mating type are required to account for the various results. The scheme differs in several respects from that devised by Oshima and his coworkers in Japan. One provocative idea, that mating type genes may be carried on episomes and inserted or exchanged at the mating type locus, prompted interested discussion.

Dr. V. G. Korolev - Mutagenic effects of ^{32}P and ^{35}S.

Both ^{32}P and ^{35}S, incorporated into cells, are effective mutagens. Approximately $1/10^6$ disintegrations of ^{32}P results in a mutation too for a red colony characteristic of ade 1 and ade 2 mutations, and 2-3% of all decays of both ^{32}P and ^{35}S lend to lethal effects. The efficiency of mutation is similar to that induced

Dr. G. F. Nesterova - Genetic Control of Killer Character in S. cerevisiae.

After reviewing progress in characterizing the double stranded RNA associated with killer character both in the USSR and US, Dr. Nesterova presented evidence for a new nuclear gene controlling the maintenance of killer, mmn2, which is linked to lys9 in chromosome XIV. The most striking observation presented was that killer character is inhibited by two unlinked dominant suppressors both of which were also ochre (nonsense) super suppressors. Amber suppressors did not effect killer. Surprisingly, the suppressors of killer do not "cure" double-stranded RNA.

Dr. A. Arcfyeva - Multiple Mutations in S. cerevisiae.

Two strains of yeast have been found in the Peterhof collection which form multiple mutations at the frequency of single mutations. In some cases as many as 5 apparently separable mutations can be induced by UV at a frequency of 10^{-4} to 10^{-5} (as opposed to an expected 10^{-20} or less). One strain a ade 1), when selected for mutations of the mating type, was found to yield cells of genotype α ADE 1 his leu2 rap (slow growth). These mutations are on at least 3 different linkage groups, but three of them [leu2, rap, mating type] are all on chromosome III. Only some mutations are observed. Whether the mutations are independent (for example, whether each leu2 mutation is a different allele) has not yet been tested. For example, when forward mutations are selected for cycloheximide resistance, no multiple mutations are found - nor when adenine prototrophs are selected directly. The genetic control of multiple mutability is complex - not monogenic. In some cases segregants which show mutability also can be induced to obtain a secondary collection of multiple mutants, but this is not true in nearly all cases.

N. G. Krasnopevtseva, M. R. Smirnov and N. G. Padkina - Studies on the formation of genes controlling the activity of exogenous constitutive acid phosphatase.

Two acid phosphatases, acid phosphatase 1 and acid phosphatase 2, were excreted into the culture medium of S. cerevisiae. These two enzymes have been purified by concentration of culture medium and by column chromatography. Acid phosphatase 1 is stable at pH 3.2, has a pH optimum of 3.6, and is heat stabled at 50°C. Acid phosphatase 2 is unstable at pH 3.2 and has a pH optimum of 4.0. Antiserum to acid phosphatase 1 also gives precipitation with acid phosphatase 2.

S. A. Koznin and M. N. Smirnov - Studies on genetic control of the synthesis of exogenous repressible acid phosphatase.

In the genetic study, acid phosphatase negative mutants have been isolated from S. cerevisiae. Of these mutants, 122 belong to PHO 1 locus, 5 in PHO 2 locus, 2 in PHO 3 locus, 2 in PHO 4 locus and 1 in PHO 5 locus. PHO 1 locus is the structural gene for acid phosphatase 1. PHO 1 and PHO 2 genes are linked. Several genes are involved in the synthesis and regulation of acid phosphatase 2. A model was presented for the synthesis, control and excretion of acid phosphatase. Dr. Oshima of Osaka, Japan has studied in detail the genetic basis for the synthesis of inducible and repressible acid phosphatases in S. cerevisiae. Dr. Smirnov should obtain Dr. Oshima's tester strains and compare the genetic properties of his mutants and his enzymes.

Ya. O. Soom, II. Polsporukob and I. A. Popopova - Methanol Utilization of Pichia pinus

These workers have isolated 57 mutants which are unable to utilize methanol. They are presently working on a genetic analysis of these mutants including recombination and complementation analysis. However, the genetic studies are not yet complete. Their future work is expected to include a biochemical analysis of these mutants.

B. Entomopathogenic Microorganisms.

R. R. Azizbekyan, B. V. Smirnov, and I. B. Mineykova (Institute of Genetics of Microorganisms, Moscow). Isolation and Characterization of New Phages of B. thuringiensis

This paper consisted of a rather brief description of several phages active on B. thuringiensis and/or other species of Bacillus. In all instances the phages were isolated from soil rather than from lysogenic strains. Two morphological types were represented among the various phages; one type having a contractile tail and the other type having a flexible noncontractile tail. Several of the phages were unstable during storage although none was shown to be specifically cold-labile.

The most interesting and novel aspect of the paper was that dealing with "polyvalent" phages, i.e., phages that were active on more than one species of Bacillus. A particularly interesting one, designated phage AR-7, was active on strains of B. subtilis, B. megaterium, B. cereus, and B. thuringiensis. Insofar as I am aware Bacillus phages with such extensive host range have not been reported previously. Such phages might prove useful in attempts to exchange plasmids among various species of Bacillus.---C. B. Thorne

B. P. Karabekov (Institute of Genetics of Microorganisms, Branch in Armenia) Behavioral Patterns of Some Phages of B. thuringiensis

The paper described results of some preliminary studies on various phages carried by strains of B. thuringiensis. The work stems from interest in lysogenic systems among strains of B. thuringiensis. The strain of variety galleriae being used commercially in insecticidal preparations was shown to carry phage only recently after a suitable indicator was isolated. The authors found at least one phage that is cold-labile and which, in this respect, appears similar to CP-51 described by Thorne (J. Virol. 2:657(1968). After 30 minutes of exposure to 0 C, only 1% of the initial viability was retained. Observations were reported on factors affecting lysis of cultures following infection by phage; e.g., older cultures do not lyse when infected with phage and in some instances cultures pass through two or three cycles of growth and lysis following infection. These observations appear to be similar to those observed in other laboratories concerned with Bacillus phages.---C. B. Thorne

M. G. Oganessian (Institute of Genetics of Microorganisms, Branch in Armenia). Isolation and Preliminary Investigation of Biochemical and Morphological Mutants of B. thuringiensis.

The synthetic medium used as minimal medium in mutant isolations was reported to be similar to that described by Nickerson and Bulla except that trace elements were omitted. The addition of glutamic acid was found to be necessary for the growth of B. thuringiensis strains. Nitrosoguanidine was the only mutagen reported to be in use. Presumably mutants could be isolated without difficulty and those that were isolated represented a wide variety of auxotrophic markers. Only about 20% of the auxotrophic mutants could be characterized by the procedures employed, and a good proportion of these were double mutants. Many of the mutants were also defective in crystal formation. An interesting finding was that several wild-type strains required nicotinic acid for growth and at the same time were unable to use sucrose. It is likely that some of the mutants that have been isolated during studies in my laboratory and the authors agreed to send me any that I request.---C. B. Thorne

I.A. Zakharov, L.V. Yurchenko - Genetical Studies on Entomopathogenic Fungus Beauveria bassiana.

A product called "Beauverin" is made in the USSR from B. bassiana and this is used rather extensively as an insecticide. The goal of the research reported by Zakharov was to isolate mutants of the fungus which had improved pathogenicity. The lab. animal used to assay pathogenicity was the fruit fly, Drosophila melanogaster. Conidiospores were treated with UV or x-rays and morphological mutants were selected. These mutants were mostly affected in size, shape, or pigmentation of the colony. Mutants were also selected for resistance to a fungicide. Auxotrophs were also selected. The result was that none of the mutants showed increased pathogenicity compared to the parent strain.

J. Rubikas, K. Sasnauskas, J. Jomantas - The Physiological Aspects of the Bacillus subtilis Sporulation Genetics.

Rubikas reviewed the physiological events known to occur during the stages of sporulation in B. subtilis . A number of mutants were selected which he believed to be affected in their response to catabolite repression. The response was measured mainly by the time of pH changes, extracellular protease production, and the formation of heat stable spores. The sites of the actual mutations were not determined.

4. Evaluation of the conference

This conference permitted the US delegation to explore in some detail the scientific activities in two specified areas of interest in the Exchange Program. Our evaluations are based upon the talks presented by the USSR scientists and extensive discussion following each, visits to several laboratories to review the quality of their research potential, discussions with senior scientists, and most important, discussions with young junior scientists particulary in the Leningrad area. Evaluation of this inter-action was summarized under areas of mutual interest, US benefits resulting from the visit and evaluation of the research potential in these two areas.

(a) Areas of mutual interest.

Considerable interest exists in both the US and USSR in the theoretical and applied aspects of genetics of yeast. In the USSR a series of polyploid hybrid yeasts Saccharomyces species used in wine production were obtained, while in the US five homothallic strains suitable for genetic analysis were obtained to study genetic factors affecting wine production. In the USSR methods to analyze the genetics of Pichia pinus utilizing methynol and Pichia guilliermondii utilizing paraffin were undertaken to study genetic control of carbon utilization. On the other hand, in the US genetic studies of Saccharomyces lipolytica utilizing hydrocarbons have been initiated. In both countries there is mutual interest in production of metobolic products. Interest in the USSR was for mutants to control enzyme acid phosphatase whereas in the US histidine, lysine, and methionine excreting mutants have been investigated. In both countries efforts to improve mutagenesis have been underway. In the USSR a new method was developed to obtain mutants arising from induced genetic instability. In the US new selective techniques to obtain spontaneous mutants were developed and, in addition, mutants affecting mitotic chromosome loss were used to obtain various aneuploid strains.

In both countries there is extensive interest in the genetics of entomopathogenic microorganisms. These efforts were directed towards seeking transducing and polyvalent phages for Bacillus thuringiensis and the isolation characterization of auxotrophic

mutants and mutants defective in spore and crystal formation. Since this has not been achieved in either country interest was directed toward exploring new approaches in order to provide a genetic vehicle and for the appropriate mutant selection.

(b) US benefits resulting from this conference.

One of the most significant developments from the meeting was the obtaining from Dr. Inge-Vechtomov of his mutant strain (D131) of S. cerevisiae which has been mutated to utilize alkanes. We had previously been exposed to this claim at the conference in Armenia a few years ago but had been skeptical about its validity. Its importance lies in the fact that at the present time we have had available Candida strains for alkane utilization and these are not acceptable as an approved yeast for single cell protein whereas S. cerevisiae is an accepted single cell protein yeast. Dr. Bassel obtained a copy of the strain which he brought back to California and Dr. Mortimer's laboratory confirmed the fact that it is a mutant of S. cerevisiae and possesses the characteristics described by the Leningrad group. In addition, Dr. Mortimer has derived additional variants of this strain which have other valuable properties. This strain holds high promise as a food yeast and is a major contribution. See table below.

Strains	Fermentation Media - B.T.B.					Synthetic +Trp, Ade, Met, Ura						
	Sucrose	Galactose	Maltose	Melibiose	α Methyl Glucoside	Glucose	Ethanol	Decane	Hexa Decane	C^{12} Alcohol	C^{12} Aldehyde	Ole Aci
1. CX39-74B	+	+	+	-	-	+	+	+	+	+	+	+
2. X2180-14	+	-	-	-	-	+	+	-	-	-	-	-
3. D131	+	+	+	-	-	+	+	-	-	-	-	-
4. D131-3-4	-	+ -	-	-	-	-	+	+	+	+	+	+
5. (IV-2)-4-6	-	-	-	-	-	+	+	+	+	+	+	+
6. (IV-2)-4-9	-	-	-	-	-	+	+	+	+	+	+	+

AGAR MEDIA, THREE DAYS INCUBATION AT 24°C

STRAINS:	SOURCE OF STRAINS	
1. CX39-74B	Berkeley	Saccharomyces lipolytica haploid
2. X2180-1A	Berkeley	Saccharomyces cerevisiae haploid
3. D131	Leningrad	Saccharomyces cerevisiae diploid, Alkane negative
4. *D131-3-4	Leningrad	Saccharomyces cerevisiae diploid, Alkane positive mutant
5. *(IV-2)-4-6	Leningrad	Saccharomyces cerevisiae diploid, Alkane positive mutant
6. *(IV-2)4-9	Leningrad	Saccharomyces cerevisiae diploid, Alkane positive mutant

*4, 5, and 6 were derived by Dr. Mortimer by strain three received from Dr. I. Vechtomov.

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Dr. Haber has obtained several important strains for the Leningrad group. The first contained a new marker on chromosome III which is near the mating type locus. This mutant affects rate and is designated rap. Since there are so few markers available near the mating type locus new mutants are of particular interest in studies of the genetics of the mating reaction. The other strains obtained were those which exhibit multiple mutations, and which were discussed at the meeting. It is not yet clear what the basis of this multiple mutation is; however, one possibility is that these strains may carry fragments of chromosome III. They also are going to be very important in studying the mating type.

A large collection of reprints were obtained during our visit to the USSR. These have been distributed to scientists in areas of interest. Where information is of sufficient importance these particular reprints will be identified and translated. During the visit we discovered that the USSR had a very extensive computer program for following publications in the US whereas we were, to a large extent, ignorant of their contributions.

As a result of our discussions several of the US delegation expressed interest and explored possibilities for more extended visits to the USSR during the coming year.

(c) Evaluation of the research in the USSR.

In evaluating the genetics in the USSR it is important to recall that although the USSR scientists appear extremely willing to cooperate, the country is recovering from a thirty year legacy of suppression of basic genetics. Nevertheless a number of differences in approach were discovered leading to a number of fruitful discussions and possibilities. With regard to yeast genetics a more classical approach is being stressed with the Leningrad scientists. Their group is well equipped to isolate and map mutants in yeast and to contribute to improved methods for mutagenesis. The younger scientists were particularly disturbed on hearing from Dr. Sherman's report that when one analyzes the amino acid sequence of cytochrome C in mutants and from that infers genetic structure, there is not a linear relationship between recombination distance and physical distance. In visitations

to the laboratories it is clear that they are not equipped to carry out similar analysis of the effect of mutations on gene products. In addition, the genes under most extensive investigation involved protein products which have not been well characterized and do not lend themselves to sequence studies although a major effort to purify the gene product of the ADE2 locus is underway.

The younger scientists readily grasped the significance of studies of this kind and appeared disturbed that saturation mapping of a gene would not yield the results that they had hoped to find. It also appeared there were limits to their ability to carry out experiments of their own wishes. It appeared that the majority of their time was on assigned projects from the Institute. The facilities at the Leningrad University were suitable for classical genetic studies but with the exception of one laboratory significantly lacking in biochemical instrumentation. Consequently it was felt among the visiting group that a more promising way for our interaction to proceed with the Leningrad group was instead of sending students to their laboratories for a short time that we send over a very small group to conduct a workshop on molecular and biochemical techniques. It appeared that they would be capable of assembling from the Leningrad area the necessary equipment for carrying out such a workshop.

In studies on Bacillus thuringiensis it appeared that major breakthroughs have not been carried out in either country at the present time. Both have an interest in applying genetics and developing it but the results to date have not as yet yielded a system for genetic analysis. The Russians contributions have largely been in the physiology of the organism, characterization of phages, and the selection of mutants in Bacillus thuringiensis. In the areas discussed there was very little new information unknown to our US representatives in this area.

5. Appendix

A. List of Participants

USSR

1.	Shenderei, E. R.	Vice Director of Glavmikrobioprom
2.	Sukhodolets, V. V.	Vice Director of Institute of "VNII-Genetika"
3.	Inge-Vechtomov, S. G.	Chief of Dept. of Genetics a.Selection, Leningrad State University
4.	Rodionova, V. G.	VNII-Genetika, Foreign department
5.	Nesterova, G. F.	Dept. of Genetics a.Selection, Leningrad State Univ.
6.	Azizbekjan, R. R.	Chief of Laboratory, VNII-Genetika
7.	Zacharov, I. A.	Chief of Laboratory, Leningrad Institute of Nuclear Energy. Acad. Sci.
8.	Korogodin, V. I.	Chief of Laboratory, VNII-Genetika
9.	Kurenkov, B. P.	Vice Chief of Foreign Relations of Glavmikrobioprom
10.	Serjegin, V. I.	Vice Chief of Technikal, Dept. of Glavmikrobioprom
11.	Smirnov, M. N.	Chief of Laboratory, Dept. of Genetics, Leningrad State Univ.
12.	Kapulzevich Ju. G.	VNII-Genetika
13.	Karabekov, B. P.	Chief of Laboratory, Charenzavan's Branch of VNII-Genetika
14.	Korolev, V. G.	Chief of Research Group, Leningrad Institute of Nuclear Energy, Acad. Sci.
15.	Melnikov, L. A.	VNII-Synthesbelok
16.	Minenkova, I. B.	VNII-Genetika
17.	Naumov, G. I.	VNII-Genetika
18.	Oganesjan, M. G.	Director of Armenian branch of VNII-Genetika
19.	Rubicas, L.P.	Chief of Molecular Genetics Group Inst. of Biochemistry Acad. Sci. of Lithuaenija

20.	Simarov, B. V.	Dept. of Genetics, Leningrad University
21.	Skladnev, A. A.	Head Chief of the Technical Department of Glavmictobrioprom
22.	Soidla, T. R.	Dept. of Genetics, Leningrad University
23.	Tolstorukov, Y. Y.	VNII-Genetica
24.	Tarasov, V. A.	Chief of the Laboratory, Institute of General Genetics Acad. Sci. USSR
25.	Lushnikov, A. A.	VNII-Synthesbelok
26.	Gradova, N. B.	Vice Director of the Institute VNII-Synthesbelok
27.	Kozhin, S. A.	Dept. of Genetics, Leningrad State University
28.	Smirnov, V. N.	Chief of Laboratory, Institute of Kardiology
29.	Soom Ja. O.	Dept. of Genetics a. Selection, Leningrad State Univ.
30.	Dmitrenko, L. V.	Director of VNII-Hydrolisis
31.	Domoradsky, I. V.	Chief of Lab, VNII-Synthesbelok

USA

1.	Bassel, Dr. John	Donner Laboratory, Univ. of California, Berkeley, Calif.
2.	Bhattacharjee, Dr. J.	Dept. of Microbiology, Miami Univ., Oxford, Ohio
3.	Fink, Dr. Gerald	Dept. of Genetics, Cornell University, Ithaca, New York
4.	Fogel, Dr. Seymour	Dept. of Genetics, Univ. of California, Berkeley, Calif.
5.	Haber, Dr. James	Rosenstiel Center & Dept. of Biology, Brandeis Univ. Waltham, Mass.
6.	Halvorson, Dr. H. O.	Director, Rosenstiel Center & Prof of Biology, Brandeis Univ., Waltham, Mass.
7.	Henry, Dr. Susan	Dept. of Genetics, Albert Einstein College of Medicine Bronx, New York
8.	Sherman, Dr. Fred	Dept. of Rad. Biology & Biophysics, Univ. of Rochester, N.Y.
9.	Thorne, Dr. C. B.	Dept. of Microbiology, Univ. of Mass., Amherst, Mass.
10.	Yousten, Dr. Allan A.	Dept. of Biology, Virginia Polytechnic State Univ. Blacksburg, Virginia

B. Program

US/USSR SEMINAR ON THE GENETICS OF YEAST AND ENTOMOPATHOGENIC MICROORGANISMS

Nov. 17	F. Sherman (Univ. of Rochester)	The Use of Chromosome C Mutants of Yeast for Elucidating the Nucleotide Sequence that Governs the Initiation of Translation
	D. Fink (Cornell Univ.)	Molecular Basis of Suppression in Yeast
	V. V. Kvasha, D. A. Gorodenin, S. G. Inge-Vechtomov (Zhdanov Leningrad State University)	Mapping of Mutations in ade2 Locus in Saccharomyces cerevisiae
	T. R. Soidla, N. P. Mikhailova (Zhdanov Leningrad State Univ.)	Interallelic Complementation in ade2 Locus and its Variability
	B. V. Simarov, A. Shauki, N. N. Khromov-Borisov (Zhdanov Leningrad State Univ.)	Mutagenic Effect of 6-Hydroxyl-aminopurine in Yeast S. cerevisiae
	V. N. Smirnov (Institute of Cardiology of USSR Academy of Sciences), L. V. Lyzlova, Ye.S. Fominykh, A. P. Surguchev, S. G. Inge-Vechtomov (Zhdanov Leningrad State Univ.)	Studies on the Mechanism of Recessive Super Suppression in Yeast
Nov. 18	H. Halvorson (Brandeis Univ.)	Studies in the Structure of Chromosome I in Yeast
	J. Haber (Brandeis Univ.)	Genetically Directed Mitotic Chromosome Loss in Yeast
	I. A. Zakharov, V. P. Stepanova, B. F. Yarovoi (Institute of Nuclear Physics of USSR Academy of Sciences)	Cytoduction in Yeast
	A. Ya. Arefyeva, S. G. Inge-Vechtomov (Zhdanov Leningrad State University)	Multiple Mutants in S. cerevisiae
	G. F. Nesterova, A. A. Filatov, A. M. Zekhnov (Zhdanov Leningrad State University)	Genetic Studies in Killing Activity Trait in S. cerevisiae
	G. I. Naumov (Institute of Genetics of Microorganisms, Moscow)	A Comparative Genetics of Homo- and Heterotallism in Yeast Saccharomyces
	V. G. Korolev, L. M. Gracheva (Institute of Nuclear Physics of USSR Academy of Sciences)	Genetic Effects of Radioisotopes P^{32} and S^{35} on Yeast

ov. 19	S. Fogel (Univ. of Cal...)	Genetics of Amino Acid Excretion in Yeast
	J. Bhattacharjee (Miami Univ.)	Feasibility of Mutation for the Procurement of High Lysine Producing Yeast
	N. G. Krasnopevtseva, M. N. Smirnov, N.G. Padkina (Zhdanov Leningrad State Univ.)	Studies on the Function of Genes Controlling the Activity of Exogenous Constitutive Acid Phosphatase
	S. A. Koznin, M. N. Smirnov (Zhdanov Leningrad State Univ.)	Studies on Genetic Control of the Synthesis of Exogenous Repressible Acid Phosphatase
	Visit to the Chair of Genetics & Selection of the Leningrad State University	
lov. 20	S. Henry (Albert Einstein College of Medicine)	Mutations in Yeast Affecting Synthesis of Membrane Lipids
	J. Bassel (Univ. of Calif.)	Genetics of Alkane Utilization in Saccharomycopsis lipolytica
	Ya. O. Soom, I. I. Tolstorukov, I. A. Popova (Institute of Genetics of Microorganisms, Moscow, Zhdanov Leningrad State University)	Genetic Studies on Methanol Utilizing Yeast Pichia pinus
	Visit to the Laboratory of Radiation Genetics of the Leningrad Institute of Nuclear Physics	
lov. 21	C. Thorne, (Univ. of Mass.)	Transducing Phages for Bacillus cereus and B. thuringiensis
	A. Yousten (Virginia Polytechnical State University)	Bacillus thuringiensis of Physiological Profile and Prospects for Genetics Exchange
	R. R. Azizbekian, V. B. Smirnov, I. B. Minenkova (Institute of Genetics of Microorganisms, Moscow)	Isolation and Characterization of New Phages of B. thuringiensis
	M. G. Oganessian (Institute of Genetics of Microorganisms, Branch in Armenia)	Isolation and Preliminary Investigation of Biochemical and Morphological Mutants of B. thuringiensis
	B. P. Karabekov (Institute of Genetics of Microorganisms, Branch in Armenia)	Behavioural Patterns of some Phages of B. thuringiensis

Nov. 21 contd.

I. A. Zakharov, E. V. Ivanchenko (Institute of Nuclear Physics of the USSR Academy of Sciences)

Genetic Control of Entomopathogenic Fungus Beauveria bassiana

J. Rubikas, K. Sasnauskas, J. Jomantas (Institute of Biochemistry of the Lithuanian Academy of Sciences, Vilnius)

The Physiological Aspects of the Bacillus subtilis Sporulation Genetics

C. US Abstracts

Genetically Controlled Chromosome Loss
Saccharomyces cerevisiae

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While normal a/α diploid strains of Saccharomyces cerevisiae do not mate with strains of either mating type, diploids homozygous for a recessive mutation appear to be bisexual, that is, able to mate with both a and α tester strains. Bisexual mating appears as a result of a high frequency (about 1 - 2%) of formation of 2n-1 aneuploid strains for chromosome III. This aneuploid formation is under the control of a single, recessive gene designated chl for chromosome loss. The chromosome loss gene is apparently centromere linked, about 20 cM from a centromere, but its assignment to a known linkage group has not been achieved.

In diploids homozygous for chl, chromosome loss to form 2n-1 aneuploids has only been demonstrated for chromosome III, and not for chromosomes V, VII and IX. However, high levels of mitotic recombination have been found for many other chromosomes, including the 2n-1 chromosome III aneuploids. The chl mutation also dramatically increases the reversion of strains disomic n+1 (a/α) for chromosome III to euploid a or α strains.

Diploids homozygous for chl are able to sporulate, although tetrad viability is often reduced. There is also an apparent decrease in meiotic recombination when tetrads from chl/chl strains are compared to chl/+ strains.

Transducing Phages for *Bacillus cereus* and *Bacillus thuringiensis*

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Previous reports from this laboratory have shown that phage CP-51, which was isolated from soil, is a generalized transducing phage for several strains of *B. cereus* and *B. anthracis*. All auxotrophic mutants tested, including a wide variety of markers, were transduced to prototrophy. Although CP-51 displayed some properties attributed to temperate phages, it was only moderately temperate and appeared not to establish a stable lysogenic relationship with any of the hosts that were tested. Phage CP-51 is unusual among phages thus far reported in that it is cold-labile. It was rapidly inactivated when stored at the usual refrigerator temperatures (2 to 4 C) and even more rapidly when exposed to 0 C. Stability was enhanced by storage at higher temperatures; the optimal temperature of those tested for maintenance of plaque-forming units was 15 C. Untreated preparations lost up to 90% of their initial numbers of plaque-forming units in 24 hours when stored at 2 to 4 C, although high titers were maintained for several days at 15 C. The loss in viability resulting from exposure to cold correlated with an increase in numbers of phage particles having contracted tails. High concentrations (0.01 M) of Mg^{2+}, Ca^{2+}, or Mn^{2+} stabilized the phage considerably, but even in the presence of these divalent cations, it was much more stable at 15 C than at 0 C.

A second generalized transducing phage for *B. cereus* strains 6464 and 569, designated CP-53, has been shown to be carried by strain 6464 in what appears to be a very stable lysogenic relationship. The two phages, CP-51 and CP-53, are apparently unrelated. CP-51 gave greater frequencies of cotransduction for linked markers than did CP-53; this is consistent with the finding that CP-51 is a larger phage than CP-53 and contains more DNA. CP-51 DNA contains about 43% guanine plus cytosine and it has 5-hydroxymethyluracil in place of thymine. CP-53 DNA contains no unusual bases and its guanine plus cytosine content is 37%.

Current studies are concerned with transduction and phage-host interactions in *B. thuringiensis*. Fourteen varieties of *B. thuringiensis*, out of 18 varieties tested, were found to be hosts for CP-51. Thus far, we have tested only one variety, *thompsoni*, for transduction with CP-51; tryptophan, leucine, and purine auxotrophs were transduced to prototrophy. In a survey of varieties for sensitivity to CP-53, we found that 4 out of 18 varieties tested served as hosts. Since CP-53 transduces susceptible strains of *B. cereus*, it will probably also transduce the susceptible varieties of *B. thuringiensis*.

Recently we have isolated a third transducing phage from soil. All 18 varieties of *B. thuringiensis* tested served as hosts for the phage. We have tentatively designat this phage as CP-54, although it is possibly a host-range mutant of CP-51. It is inactivated with CP-51 antiserum and is even more cold-labile than CP-51. Its action on the various strains varies from moderately temperate to very virulent. Thus far we have tested only two varieties, *finitimus* and *alesti*, for transduction. Auxotrophic mutants including those requiring tryptophan, nicotinic acid, or methionine were transduced to prototrophy.

Bacillus thuringiensis - A Physiological Profile and Prospects for Gene Transfer

Allan A. Yousten

Much less is known about the nutrition, metabolism, and physiology of sporulation in Bacillus thuringiensis than in some other bacilli. This bacterium resembles B. cereus in a number of respects and grows slowly in glucose-salts defined media if glutamate is present. Vegetative cell metabolism of glucose is primarily via the Embden-Myerhof-Parnas pathway. Enzymes of the tricarboxylic acid (TCA) cycle are derepressed following exponential growth and this pathway presumably provides energy during sporulation. TCA cycle mutants have not yet been isolated to examine the relationship between sporulation and the TCA cycle. Extracellular amylase and a metal chelator-sensitive protease appear in late exponential and early stationary phase, respectively. Synthesis of these enzymes is dependent upon different combinations of cations in the growth medium. The chemical composition of the paraspore (crystal), the timing of protein synthesis, and the appearance of toxicity will be reviewed. At the present time transduction appears to offer the best opportunity for achieving gene transfer among these bacteria. Although B. thuringiensis is lysogenic, the phages it carries appear less suitable for transduction than phage CP-51. Phage CP-51 reacts in B. thuringiensis in much the same way it did in B. cereus where Dr. Curtis Thorne first described it as a transducing agent.

Biosynthesis and Production of Lysine in Yeast

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Genetic as well as biochemical basis for the synthesis and the increased production of lysine were investigated in yeast, Saccharomyces cerevisiae. Four chemical agents, EMS, MNNG, NA, 1CR-170, as well as UV were used to procure lysine auxotrophs from X2180-1B (α) of S. cerevisiae. A total of 2053 lysine requiring mutant clones were isolated from many independent treatments and by nystatin enrichment technique. Mutants were classified into various functional groups on the basis of complementation analysis. Mutant clones were unevenly distributed into different loci, with only 2 isolates belonging to lys5 locus, and 918 isolates belonging to lys4 locus. A total of 44 isolates complemented with all 14 tester strains. Seven lysine loci are mapped on six different linkage groups of S. cerevisie.

Biochemical steps involved in the synthesis of lysine were elucidated on the basis of a. feeding of specific precursor of lysine to support nutritional requirement of lysine auxotrophs, b. accumulation of one or more precursor(s) of lysine in specific lysine auxotroph, c. lack of a given enzyme activity in specific lysine auxotroph, and d. feedback regulation of specific enzymes by lysine and analogs of lysine.

Fifty mutant clones resistant to thialysine, an analog of lysine, were procured by treatment of X2180 with MNNG. Five of these mutants produced significantly high level of lysine in the culture supernatant compared to the parent strain. Extent of lysine excretion by these analog resistant mutants were determined by microbiological assay, chemical assay, and radioactive assay procedures.

Dr. John Bassel
Donner Laboratory
University of California
Berkeley, California

Strains of the hydrocarbon utilizing yeast, Saccharomycopsis lipolytica, have been developed which show a high frequency of sporulation and ascospore germination frequencies of about 85%. With these strains it is possible to do both random spore and tetrad genetic analyses. By means of ultra-violet mutagenesis a number of mutant phenotypes have been isolated. These include auxotrophic, color, morphological and hydrocarbon negative mutants. A red mutant has been found to excrete large quantities of protoporphyrin IX. This mutation shows Mendelian segregation and is recessive. A number of hydrocarbon negative mutants have been isolated. Most of these have been put into crosses. These mutations are recessive to wild type and segregate in meiotic ascospores. Feeding tests have been conducted in order to tentatively correlate the genetic blocks with known steps in hydrocarbon metabolism.

Dr. Harlyn O. Halvorson
Director, Rosenstiel Basic
Medical Sciences Research Center
Professor of Biology

"STUDIES ON THE STRUCTURE OF CHROMOSOME I IN YEAST"

a. Mapping of the rRNA Genes to Chromosome I

The one hundred and forty genes for ribosomal RNA (rDNA) in Saccharomyces cerevisiae occur in clusters of from ten to thirty cistrons. Approximately one hundred rDNA genes have been mapped to chromosome I by directly comparing the amount of rDNA in a strain monosomic (2n-1) for chromosome I with a related diploid (2n) strain. The monosome showed an approximate thirty percent decrease from the diploid in both the amount of heavy satellite DNA and hybridizable rRNA. It is proposed that three to ten clusters of rDNA are located throughout chromosome I.

To study the arrangement of the 5S RNA genes in relation to the 18S and 26S rRNA genes we examined the number of 5S RNA genes that map on chromosome I. The amount of 5S RNA hybridized to DNA isolated from a strain monosomic (2N-1) for chromosome I was compared with DNA isolated from a related diploid (2N). The monosomic strain showed approximately 25% less 5S RNA hybridized than the diploid. Therefore, about 50% of the 5S RNA genes are associated with chromosome I. As a control we determined saturation hybridization levels with 5.8S RNA, an RNA species derived from the 35S rRNA precursor. The monosome for chromosome I gave approximately 27% less 5.8S RNA hybridized than the diploid, an amount equivalent to 54% of the rRNA genes mapping on chromosome I. Therefore, about the same number of 5S RNA genes are on chromosome I as 18S and 26S rRNA genes. This confirms the earlier observation of the physical linkage of the 5S genes to the 18S and 26S rRNA genes. These findings are in contrast to other eucaryotes studied where the 5S genes are unlinked to the 18S and 28S rRNA genes.

b. Saturation Mapping of Chromosome I

Although 60-70% of the rRNA cistrons are clustered on chromosome I, the physical characterization of nuclear DNA indicates that this repetitive DNA sequence is not present as a continuous DNA component. The rDNA satellite is evident only after the shearing of nuclear DNA, indicating that rRNA clusters are spaced by a region higher in AT bases than rDNA. These observations pose the question of the composition and order of the genetic information on chromosome I and its relationship to rDNA and nucleolar structure and function. I have used a genetic approach to isolate, characterize and study the arrangement of mutants mapping on chromosome I in relation to the rRNA genes. We have started to saturate the genetic map of chromosome I with temperature-sensitive mutations. Since rRNA genes are 140-fold reiterated, any lesion in a single rRNA gene should not be expressed as a mutation. Thus, the genetic loci for rRNA would be mutationally "silent" and the gene loci surrounding the "silent" region will be the borders for an rRNA gene cluster. The size of an rRNA gene cluster can be predicted

in terms of recombination distance by correlating genetic and physical distances. From the genome size of yeast and the haploid DNA content, 1 centimorgan can be claculated as 3×10^6 daltons of duplex DNA, or approximately 3/5 the size of a single rDNA gene. From the above considerations and from the physical measurements on the rDNA, rDNA clusters should have a length between 20 and 60 centimorgans. The saturation mapping of chromosome I should provide a test of this model for the arrangement of rDNA genes on chromosome I. By mapping the site of structural genes on chromosome I, I expect to establish the borders of the rRNA gene clusters.

I have developed a method which selects for mutants mapping on chromosome I which utilizes the strain monosomic (2n-1) in that chromosome. This strain is diploid for all chromosomes except chromosome I which is haploid. The selection for mutants is based on the assumption that recessive mutations on chromosomes 2-17 would be complemented by their homologous alleles. Only mutations on chromosome I or dominant mutations on other chromosomes would show a mutant phenotype. A number of mutations have been isolated by EMS mutagenesis and have been shown to map on chromosome I by genetic mapping techniques. I have carried out a large scale mutant hunt isolating 200 thermosensitive mutants which we are currently mapping.

c. Ribosomal DNA Magnification

A strain monosomic (2n-1) for chromosome I contains approximately 30% less rDNA than a related diploid (2n). We have found that when two clones of the monosomic strain were repeatedly subcultured for over one year on agar plates, the level of rDNA as determined by saturation DNA-RNA hybridization had increased from 1.6% to the wild-type level of approximately 2.2%. The original strain which was stored on silica gel for the same period remained unchanged. The subclones which contain the increased levels of rDNA (HOH-3mag$_1$ and HOH-3mag$_2$) appear to be stable. They are still monosomic for chromosome I as evidenced by the characteristic 2:2, viable:inviable segregation upon meiosis due to segregation of the null chromosome into two nonviable spores and the hemizygosity of the ade 1 gene (a chromosome I marker). DNA-RNA hybridization experiments with several viable spores from HOH-3mag$_1$ show that the extra rDNA is segregating as a Mendelian allele unlinked to ade 1 indicating it is on a chromosome other than chromosome I. The presence of several tetrads where the rDNA exhibits second division segregation indicates that it is not tightly contromere linked. In summary, the rDNA level in a strain initially deficient in rRNA genes has been magnified to the wild-type level. This phenomenon may be analogous to the reported magnification of rDNA in Drosophila melanogaster bb mutants which are deficient in these genes.

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ABSTRACT

MUTATIONS IN YEAST AFFECTING THE SYNTHESIS OF MEMBRANE LIPIDS - Fatty acid synthetase and inositol synthetase mutants of yeast are both defective in the synthesis of essential membrane phospholipids under conditions of deprivation for their respective auxotrophic requirements. In the absence of exogenous fatty acid, fatty acid synthetase mutants are deficient in the synthesis of all fatty acid containing lipids including phospholipids, whereas inositol starved inositol synthetase mutants are defective only in the synthesis of inositol containing phospholipids. Cells of both types of mutants, however, rapidly lose viability when deprived of their respective auxotrophic requirements. The kinetics of viability loss are similar in both mutants, suggesting that failure to make inositol containing lipids may be the cause of cell death in both cases. In both fatty acid and inositol starved cells, macromolecular synthesis continues at rates comparable to unstarved cells for 30 - 60 minutes after cell division has ceased. If macromolecular synthesis is blocked during fatty acid or inositol starvation, the cells remain viable. - This observation has been used to devise highly efficient selection techniques using either the fatty acid or inositol requiring strains. A mutation reversibly blocking macromolecular synthesis during fatty acid or inositol starvation is expected to protect cell viability. Thus, mutants such as amino acid, purine or pyrimidine auxotrophs and certain types of temperature and antibiotic sensitive mutants are readily selected by appropriate manipulation of inositol or fatty acid requiring strains. Enrichment in excess of 10,000 fold for certain classes of mutants has been achieved using this technique.

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THE USE OF ISO-1-CYTOCHROME *c* MUTANTS OF YEAST FOR ELUCIDATING THE NUCLEOTIDE SEQUENCES THAT GOVERN INITIATION OF TRANSLATION.

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The AUG codon for initiation of translation was previously identified from altered amino acid sequences of iso-1-cytochromes *c* in revertants of certain *cyc1* mutants of the yeast *Saccharomyces cerevisiae*. Mutants with alterations of the normal AUC initiation codon gave rise to revertant iso-1-cytochromes *c* by the formation of abnormal initiation codons corresponding to residue positions -2 and 4, thus establishing that initiation of translation could occur at least at the three sites -2, -1 and 4 indicated below:

```
      -2  -1   1   2   3   4   5
      NNN AUG ACU GAA UUC AAC CCC
          (Met)Thr-Glu-Phe-Lys-Ala-
```

However reversion by abnormal reinitiation was not uncovered after examining proteins from hundreds of revertants of mutants which contain either an ochre mutation or a frameshift mutation corresponding to position 2. The lack of reinitiation in these revertants which, of course, contain the normal AUG initiation codon, prompted us to systematically investigate defined mutants for their ability to initiate translation at positions -1 and 4. An unprecedented degree of genetic manipulation of nucleotide sequences was possible since a large number of characterized mutants are available and since procedures exists selecting strains either having or lacking iso-1-cytochrome *c* activity. Special attention was given to ten different mRNA sequences that included all combinations of GAA (glutamic acid), UAA (ochre) or GA (frameshift) at position 2, with AUG codons at either or both positions -1 and 4. The mutant sequences were constructed by recombination and mutation and were unambiguously verified by amino acid sequences of iso-1-cytochromes *c*. The present or absent of iso-1-cytochrome *c* in strains having these nucleotide sequences suggest that each mRNA sequence can have only one site for initiation of translation and that initiation is not disrupted by nonsense or frameshift mutations in front of the AUC initiation codon. Most relevant are the following two sequences in the strains *cyc1-9* and *cyc1-341* that lack iso-1-cytochrome *c*, the sequence in the strain *CYC1-341-D* that contains iso-1-cytochrome *c*, and the sequence in the strain *CYC1-340* that appears to contain only one kind of iso-1-cytochrome *c*:

```
cyc1-9        Met-Thr
              AUG ACU GAA UUC AAG GCC

cyc1-341      Met-Thr
              AUG ACU UAA UUC AUG GCC

CYC1-341-D                    (Met)Ala-
              AUA ACU UAA UUC AUG GCC

CYC1-340      (Met)Thr-Glu-Phe-Met-Ala-
              AUG ACU GAA UUC AUG GCC
```

These findings are consistent with the view that yeast cannot form polycistronic messages and that translation is fundamentally different in yeast and *E. coli*, which may represent differences between eucaryotes and prokaryotes (Supported by NIH Grant GM12702 and by ERDA, Report No. UR-3490-740).

D. Memorandum

of US/USSR workshop on Yeast Genetics and Entomopathogenic organisms conducted in accordance with the programme of Scientific and Technical Cooperation on project number 3, "Molecular biology of industrial microorganisms".

From 16-22 November 1975 a seminar was held in Leningrad in accordance with the plan of cooperation in project number 3. A list of participants in the meeting is attached (Appendix 1).

During the meeting scientific reports were given by scientific participants by the US and USSR. Twenty-seven reports were given, including 10 from the US and 16 from the USSR. The list of the reports and their authors is attached (Appendix 2).

During these meetings discussions were held between the two scientific delegations to assess the progress of the program and to explore areas in which genetic studies in yeast and entomopathogenic organisms could be extended to improve industrial production. During the period of US/USSR cooperation there has appeared a number of areas of common interest in both theoretical and applied genetics of yeast. In particular, the following main results were obtained.

In the USSR a series of polyploid hybrid yeasts Saccharomyces sp. used in wine production were obtained. In the USA five homothallic stamms scutable for genetic analysis were obtained to study genetic factors affecting wine production.

In the USSR methods to analyse the genetics of Pichia pinus utilizing methanol and Pichia guilliermondii utilizing parafin in order to study the genetic control carbon utilization. In the USA genetic studies of Saccharomycopsis lipolytica utilizing hydrocarbons were initiated. One mutant strain [illegible] protoporphyrin IX is of special interest.

D. Memorandum

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In the USSR mutants were obtained which exerete the enzyme acid phosphatase and in the USA mutants exereting histidine, lysine and methionine were found.

In the USSR, new method were developed to obtain mutants arising from induced genetic instability. Also, the mutagenic action of the purine amalog 6-hydroxyaminopurine was demonstrated. In the USA new selective techniques to obt in spontaneous mutations were developed. Also mutants affecting metabolic chromosome loss were used to obtain aneuploid strains.

Both sides note that these results are of great practical interest.

During the seminar preliminary results of studies in the area of genetics of entomophathogenic microorganisms were presented. Among the topics discussed primarily were results of sutdies on transducing and polyvalent phages for B.thuringiensis and the isolation and characterisation of auxotrophic mutants and mutants defective in spore and crystal formation. The data suggest that it will be possible for the genetic studies to lead to an understanding of the process of synthesis of the toxin crystal. The most promising directions for American-Soviet cooperation in this area were discusied and defined. The sides consider that at present efforts are to be concentrated on development particular aspects of B.thuringiensis genetics and on physiological, biochemical and genetic studies of spore and crystal formation. These discussions included future plans for the exchange of techniques strains, exchange of personnel and future meetings. Details concerning the yeast program and the entomopathogenic program are included in appendices 3 and 4.

During their stay in the USSR the US delegation was given the oppotunity to visit the Laboratory and faculty of the Department of Genetics and Selection of the Leningrad State University

and the Laboratory of Radiation Genetics of the Leningrad Institute of Nuclear Physics.

It is recognized that the goals of the US/USSR program have been to apply genetic techniques to problems of industrial importance. In the last 18 months the following areas of success could be identified.

(I) . A survey of industrial yeast the California wine industry revealed 5 homothallic strains ammenable to genetic analysis. These bill be examined in depth regarding the synthesisand activity of the alcohol dehydrogenase enzyme. These strains will provide foundation materials for important studies of alcohol production and flavor.

(2). A red mutant of Saccharomycopsis lipolytica was found to excrete protoporphyrin IX in high yield.

(3). Mutants have been isolated for the production and excretion of histidine and lysine in laboratory scale. In addition mutants for the excretion of cystine, homocysteine, arginine, uracil and methionine have been isolated and characterised.

Further details were discussed concerning plans for meetings in 1976. Dr.Halvorson extended an invitation for a USSR delegation to participante in the ASM Conference on Genetics and Molecular Biology of industrial Microorganisms at Orlando, Florida Feb.15-18, 1976. The USSR side informedthe US delegation about organisation for a seminar on genetics of cellulase andamino acid production by mixroorganisms to be held in September or October 1976, probably in Armenia. The US side confirmed their plans to held a seminar on the genetics of yeast anden tomopathogenic bacteria in November 1976.

These proposals will not be in effect until approved by the Tomt US/USSR Working Group.

Completed this day 22 November 1975. in the City of Leningrad both in English and in Russian, the two texts being anthentic.

for the US
Dr.H.V.HALVORSON
Coordinator of Projects 3

Signed by Dr.J.HABER, on behalf of Dr.HALVORSON.

for the USSR
Dr.V.SUKHODOLETS
Coordinator of Project 3

Suggestions of working subgroups to solidify the collaborative program.

Subgroup: Yeast genetics.

Participants: USA - Dr.T.Bassel, Dr.S.Fogel, Dr.J.Haber, Dr.S.Henry, Dr.F.Sherman, Dr.G.Fink, Dr.H.Halvorson, Dr.T.Battachargee

USSR- Dr.V.I.Korogodin, Dr.Ju.G.Kapulzevich, Dr. I.I.Tolstorukov, Dr.G.I.Naumov, Dr.S.G.Inge-Vechtomov, Dr.G.F.Nesterova, Dr.Ja.O.Soom, Dr.V.N.Smirnov, Dr.M.N.Smirnov, Dr.I.A.Zacharov.

In addition to the decisions made during the meeting of both sides in the USA 1975, an agreement was reached concerning a collaborative investigations in the following fields:

Topic 1. Genetics control of utilization of different carbon sourses.

Participants: USA - Dr.Bassel, Dr. Fields, Dr.R.Needelman, Dr.H. Halvorsen, Dr.N.Eaton.

USSR - Dr.G.I.Naumov, Dr.I.I.Tolstorukov, Dr.S.G. Inge-Vechtomov, Dr.Ja.O.Soom.

USA : Study of genetic control of alkane utilization in Saccharomycopsis lipolitica

USSR: Genetic mapping in Pichia pinus and searching for methanol utilizing genes.

USSR : Improvement of methods and use of genetic analysis in the yeast Pichia guillermondii and Saccharomyces sp. utilizing N-alkanes.

USA-USSR: Study of structure and function of maltose genes.

Suggestions of working subgroups to solidify the collaborative program.

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USSR : Improvement of methods and use of genetic analysis in the yeast Pichia guillermondii and Saccharomyces sp. utilizing N-alkanes.

USA-USSR: Study of structure and function of maltose genes.

The Laboratory of genetics of Plant Material Hydrolysis may accept one visiting scientist in 1977. The results of this work will be used to develop new, high productive strains of yeast utilizing different carbon sources.

Topic 2. Mitotic instability in yeast.

Participants:

USA : Dr.R.K.Mortimer, Dr.J.Haber, Dr.S.Fogel, Dr.D.Campbell.

USSR :Dr.V.I.Korogodin, Dr.Yu.G.Kapultevich, Dr.I.I. Tolstorukov.

USA - Supply of marked strains and strains aneuploid for different chromosomes.

USSR - Obtaining of Instable clones of diploid yeast after irradiation and their genetic analysis.

USA-USSR - Study of spontaneous and induced mutability in strains aneuploid for different chromosomes.

In order to carry out this topic it is necessary to send one visiting scientist to Dr.Haber's laboratory in the USA for 3 months in 1976.

Accepting an American visiting scientist in Dr.V.I.Korogodin's laboratory in 1977 is necessary.

The data reveived will be used for the developing of new methods of selection of yeast industrial strains.

Topic 3. Genetics of the sexual process in the yeast -Saccharomyces.

Participants:

USA: Drs.R.and M.Esposito, Dr.Mckay, Dr.J.Haber, Dr.G.Fink.

USSR :Dr.G.I.Naumov, Dr.S.G.Inge-Vechtomov, Dr.I.A.Zacharov, Dr.I.I.Tolstorukov.

USA-USSR : Genetic instability of chromosome III of S.cerevisiae.

USA-USSR : Genetic control of mating, diploidization and sporulation.

The results will provide the theoretical basis for improvement the industrial strains by hybridisation methods.

Topic 4. Genetics of radiosensitivity.

Participants:

USA : Dr.R.K.Mortimer

USSR : Dr.I.A.Zacharov, Dr.V.I.Korogodin.

USA : - Allelism of mutations of radiosensitivity of different origin.

USSR : Pleiotropism of mutations of radiosensitivity.

USA-USSR : Comparative study of mutants of radiosensitivity of different origin.

The results of this work will be used for improvement of methods in yeast selection by use of mutagenic factors.

Topic 5. Genetic control and regulation of resistance to heavy metals.

Participans:

USA : Dr.S.Fogel

USSR : Dr.T.R.Soidla, Ya.O.Soom.

USA : Identification of the relationship of cystein biosynthesis and copper resistance. Analysis of suppressors action on auxotrophic requirements and resistance patterns.

USSR : Study of heavy metals action on the interallelic complementation.

USSR : Comparative study of heavy metals resistant mutants in Candida, Pichia and Saccharomyces yeasts.

It is expected that the study will produce result's having theoretical implications to the development of models for regulation of metabolism and transport in industrial microorganisms.

Topic 6. Mutagenesis, mutant selection and fine structure mapping.

Participants:

USA : Dr.J.Bassel, Dr.F.Sherman, Dr.G.Fink, Dr.S. Henry, Dr.R.Mortimer,

USSR : Dr.B.V.Simarov, Dr.N.N.Khromov-Borisov, Dr.S.G. Inge-Vechtomov, Dr.T.R.Soidla, Dr.Yu.G.Kapulcevich.

USA : Molecular characterization of mutants induced by various mutagens.

USSR: Applying the methods developed to selection problems.

USA-USSR - Inducing mutations by various mutagens and their genetic characterization.

USA-USSR - Recombinational fine structure analysis.

In order to carry out this topic it is necessary to exchange the following strains and methods. Dr. S.Henry will provide protocol for mutant selection utilizing inositolless death", and inositol requirubg strains. Dr.J.Bassel will make available the facilities of yeast Genetic Stock center in Berkeley. Dr.Khromov-Borisov will provide a high-precision method for estimating frequencies. Both sides will provide each other the mutants induced by various mutagens for mutual genetic testing.

The results of this work will be used in creating better methods for mutagen treatment and genetic analysts in microorganisms.

Topic 7. Genetic control of translation and transctiption in yeast.

Participants :

USA - Dr.H.O.Halvorson, Dr.G.Fink.

USSR - Dr.S.G.Inge-Vechtomov, Dr.V.N.Smirnov.

USA-USSR - Study the protein synthesis in translational mutants using cell-free system with various mRNA preparations.

It is hoped that this study would clarify the role of yeast ribosomes and their components in the control of translation of nonsense codons in encaryiotes.

It is expected that data will be obtained which can be used as the theoretical basis in the development of new techniques for the estimation of microheterogenecity in proteins of industrial strains of yeast.

Topic 8. Genetic control and regulation biosynthesis of enzymes and metabolites.

Participants:

USA - Dr. G.R.Fink, Dr.T.R.Bhattacharjee, Dr.S.Fogel, Dr.S.Henry, Dr.C.E.Ballou

USSR : Dr.M.N.Smirnov, Dr.S.G.Inge-Vechtomov.

USA : Selection of the mutant strains with altered cell wall structure and strains with altered carbohydrate synthesis.

USA : Investigations regardship the genetic control of synthesis and excretion of important aminoacids, for example, histidine, lysine witb be carried out in the specific laboratories.

USSR : Investigation of acid phosphatase excretion through the cell of different mutants will be performed in the USSR.

It is anticipated that the collaborative investigations in this topic will lead to a better understanding the genetical control of production and excretion of useful metabolites in yeast for possible application in the microbiological industry.

Topic 9. The study of different plasmids in yeast.

Participants:

USA : Dr.G.Fink

USSR : [illegible] Nesterova, [illegible]

USA : The study of nucleic acids of yeast plasmids.

USSR : The effect of different growth conditions on plasmids and virus particles and the comparative genetics of killen strains of different origin.

USA - USSR : The study of transmission of plasmids and of transcription products of plasmid genomes.

The data will be used for the development of methods for identification of virus infections in yeast and for the construction of competitive yeast strains with special plasmids.

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TAGS: CVIS UR (AFRIKYAN, EVRIK GEGAMOVICH)
SUBJ: VISAS SPLEX: MICROBIOLOGICAL MEANS OF COMBATING AGRICULTURAL PESTS
VISAS DONKEY CHIPMUNK
1. AFRIKYAN, EVRIK GEGMOVICH
14 MAY 1925 YEREVAN
DIRECTOR, INSITUTE OF MICROBIOLOGY, ARMENIAN ACADEMY OF SCEINCES
2. ALESHINA, OL'GA ALEKSANDROVNA
8 APR. 1921 MOSCOW
DIRECTOR, ALL UNION RESEARCH INSITUTE OF MICROBIOLOGICAL MEANS OF PLANT PROTECTION AND BACTERIAL PREPARATION
3. CHEREMUKHIN, IVAN KUZ'MICH
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5. SMETNIK, ANATOLIY IVANOVICH
14 AUG. 1937 CHERNIGOV
DEPARTMENT CHIEF OF CENTRAL ADMINISTRATION OF PLANT PROTECTION, USSR MINNISTRY OF AGRICULTURE
6. ZHDANOV, VIKTOR GRIGOR'YEVICH
6 OCT. 1932 MOSCOW
DEPUTY DIRECTOR, ALL UNION RESEARCH INSITUTE OF GENETICS AND SELECTION OF INDUSTRIAL MICROORGANISMS
7. ETD DEC. 5, STAY 14 DAYS.
8. TO MEETING ON MICROBIOLOGICAL MEANS OF COMBATING AGRICULTURAL PESTS. SCIENCE AND TECHNOLOGY AGREEMENT. CONTACT WILLIAM ROOT, DEPARTMENT OF STATE OES/SCI/SEP. MATLOCK

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TELEGRAM

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MATLOCK

UNCLASSIFIED

S&T: Microbiology: Pests 01.0705

Itinerary for Soviet Delegation

January 18, 1976, at 1830 - Arrive NYC via SU-311
" International Hotel, JFK
Will be met by Watkins, Acker, or Shoemaker of American Society of Microbiology and will be accompanied by one of these three during entire trip.

January 19 at 1050 - leave NY, United 59
1215 - arrive Chicago, O'Hare

Abbott Labs - Dr. T. Couch and/or Dr. Ed Crovetti

ISM report 1/7/76

January 21 at ~~2010~~ -- leave Chicago, O'Hare, ~~AL 47~~ TWA 337
~~2236~~ - arrive San Francisco

Department of Entomology, U. of Cal. at Berkeley - Dr. Y. Tanada

January 26 at 1235 - leave San Francisco, AL 140

1740 - arrive Dallas
2040 - leave Dallas, Braniff 41
2150 - arrive Brownsville, Texas

USDA Lab, Brownsville - Dr. H. Dulmage

January 28 at 1540 - leave Brownsville, Braniff 292
1650 - arrive Dallas
1720 - leave Dallas, Braniff 112
2130 - arrive Washington, National

1/28 - 1/31 Park Central

Insect Pathology Lab, Beltsville - Dr. A. Heimpel

February 1 at 1635 - leave Washington, Dulles, TWA 900
1753 - arrive NYC, JFK
2030 - leave NYC for Moscow, SU 312

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23 APR 1976

SCIENCE & TECHNOLOGY (01.07)

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STATINTL

Report of

US/USSR Working Group on the Production of Substances by Microbiological Means

Moscow - September 18-27, 1975

FILE (01.07) Microbiology

December 2, 1975

Dr. J. M. Leise
Senior Staff Associate, MPE
Chairman, US Side of the US/USSR
Joint Working Group on the
Production of Substances by
Microbiological Means
National Science Foundation
Washington, D. C. 20550

Dear Dr. Leise:

The following is a report on the trip to Moscow and Leningrad, September 18 to September 27, 1975.

On September 22, 23, 24 we were in Moscow to hold meetings on the Exchange Program in Genetics of Industrial Microorganisms. I was a member of the U. S. Working Group and served in place of H. O. Halvorson as coordinator for Project 3. The meetings of the Working Group proceeded well and the outcome is represented by our final report. I experienced only excellent hospitality on the part of our hosts. Our accommodations were excellent, transportation was punctual, meals were good, and entertainment was first-rate. On September 25, we traveled to Leningrad by train. In Leningrad, I visited the Department of Genetics in company with Dr. Sukhodolets. I have many professional acquaintances in this department and we had a fruitful day exchanging information. My principal contact was Dr. Inge-Vechtomov, who had spent 3 months in my labroatory in 1968-69. He hosted me to a dinner in his home. I returned to Moscow by plane with Dr. Sukhodolets and on Friday, September 26 I visited the Institute of Molecular Biology and the Institute of Genetics. I met with Academician Engelhardt at the Institute of Molecular Biology.

My principal negative reactions to the visit stemmed from the fact that part of our delegation was kept in a state of uncertainty because of a sudden cancellation of their plans to visit Kiev. Only "brinksmanship" let to a reversal of this decision. On my own part, I held out for free exchange of

microbial cultures and this led to considerable consternation on the part of the Soviet members of the working group. I still support the concept of scientific exchange, however.

Sincerely,

Robert K. Mortimer

Telephone Area Code 617
253-2126

MASSACHUSETTS INSTITUTE OF TECHNOLOGY
Cambridge, Massachusetts 02139
U. S. A.

Department of Nutrition and Food Science

October 8, 1975

TO: Dr. Joshua M. Leise
National Science Foundation
Washington, DC 20550

FROM: Daniel I.C. Wang
MIT
Cambridge, MA 02139

SUBJECT: Trip Report to the USSR

The following report summarizes the activities resulting from the trip to the Soviet Union from September 21, 1975 through September 30, 1975.

A. Institute of Protein Synthesis, Moscow, USSR
(US Team: Leise, Tsao, Wang, Arminger, Field, Mortimer and Humphrey)

This writer was able to meet for the first time the coordinators of Project No. 1. Dr. Alred N. Grigorian, who was originally reported to be the coordinator of Project No. 1, was absent and was reported to be on "vacation." In his place a new coordinator, Dr. N.B. Gradova, Institute of Protein Synthesis, was appointed to be my contact. Dr. Gradova is in charge of the laboratory of physiology and biochemistry of microorganisms at this Institute. Her exact name, address and telephone number are listed below.

Dr. N.B. Gradova
All-Union Institute for Protein Synthesis
VNII Sintezbelok
27, Bol. Kommisticheskaya ul.
Moscow, USSR

Telephone number: 272-65-84

A second coordinator on Project No. 1 was also present at all of the meetings. This person was Dr. V.K. Eroshin, who had been on their negotiations on Project No. 1 several years ago. Dr. Eroshin's address and telephone number are shown below.

Dr. V.K. Eroshin
Institute of Biochemistry and Physiology of Microorganisms
USSR Academy of Sciences
Pustchino, Moscow Region
USSR

Telephone number: Serpukhov 3-08-88

During Monday, September 22 through Wednesday, September 23, 1975, the major portion of our meetings with Drs. Gradova and Eroshin were spent preparing a summary of our research and updating the future working program. In addition, a substantial amount of time was devoted to the planning of the upcoming conference on Project No. 1 in the U.S.A. It was agreed verbally that the conference will be held December 16 through 18, 1975. Depending on the availability of air flight frequencies in December, it was tentatively planned that the Soviet delegation leave the Soviet Union on December 14 and return on December 24, 1975. A tentative program of their presentations was given to the U.S. coordinators and is attached to this report as Appendix A. In addition, the Soviets have asked to visit the following places during their visit in December:

1. Romicon, Woburn, Massachusetts:

Romicon is a membrane company that engages in the industrial application of ultrafiltration membranes for protein recovery.

2. Gaulin Company, Everett, Mass.:

Gaulin Company manufactures milk homogenizers which are used for the disruption of microorganisms for the release of intracellular protein.

3. Institute of Gas Technology (IGT), Chicago, Illinois:

Institute of Gas Technology had carried out research on the use of gaseous hydrocarbons (methane) for the production of single-cell protein. The U.S. coordinators informed the Soviet scientists that IGT no longer performs research in this area. The Soviets then felt it was not worth visiting in view of these circumstances.

On Wednesday, September 24, 1975, a short visit through the laboratories at the Institute of Protein Synthesis was conducted. Dr. N.B. Gradova served as the host for this visit. Several laboratories at this Institute were visited and included:

Laboratory for Physiology and Biochemistry: strain selection
Laboratory for Biochemistry
Analytical Laboratory
"Pilot plant" for protein isolation
computer-coupled fermentor

The laboratory for physiology and biochemistry consisted of two small laboratories equipped rather modestly with the conventional equipment. The functions of these laboratories are to isolate microorganisms which utilize liquid and gaseous hydrocarbons as substrates for single-cell protein production. In addition, organisms which have capabilities of utilizing alcohols (ethanol and methanol) are also screened and isolated in these laboratories. During dis-

cussions with Dr. Gradova, it was indicated that research on the microbial utilization of cellulose is also being conducted, but not at this Institute. It was indicated by Dr. Gradova that cellulose research is being pursued at Krasnodar and Dr. Gradova seemed somewhat disturbed and reluctant to discuss this subject in detail.

At the laboratory on biochemistry, we were given a lecture with graphs and figures on the mechanism of hydrocarbon uptake by microorganisms. The laboratory was again modestly equipped compared to our standards.

The analytical laboratory in support of the research was better equipped. The instruments were mostly from the U.S. or Germany. Typical instruments include Beckman amino acid analyzer, flame ionization spectrophotometer, infrared instruments for determining benzyl pyrene concentration, and a gaseous method for determining nitrogen in single-cell protein. The latter instrument was interesting since it is capable of performing nitrogen analysis through combustion of the sample and the nitrogen content determined through volume displacement by N_2. It was indicated that very small samples (~1 mg) can be employed and approximately 7 minutes are required per sample.

A "pilot plant" for protein hydrolysis and isolation was shown to us at this Institute. The plant consisted of 4 or 5 agitated reactors which are used to perform the hydrolysis. A small scale continuous flow Westphalia centrifuge is used for solid removal. There was also a Romicon ultrafiltration unit which appears to contain 10 to 20 square feet of membrane area in this plant. By the appearance of the membrane unit, it does not appear to be operational or ever operated. As a matter of fact, the entire plant appears to be non-operational and has not been operated for some time.

The computer-coupled fermentor designed and constructed by Fermentation Design of U.S.A. was also located at this Institute. Although the fermentor and the associated equipment including the computer are all connected, it was our understanding that the interface is not working. They are waiting for the service people to correct the malfunctions.

Lastly, a small laboratory containing two 5-liter fermentors (appeared to be USSR design) were in operation. It was quoted by our hosts that these fermentors are totally instrumented for computer control. This writer has grave doubts as to their claims. The only visible instruments were CO_2 (gaseous), O_2 (gaseous), pH, temperature and dissolved oxygen. Furthermore, the gaseous CO_2 and O_2 analyses were not in operation even though a fermentation was in progress. The pH controller indicated a control capability of ± 1.0 pH unit.

The overall impression as to the quality of their research at this Institute of this writer is at best modest. Although the individuals encountered quoted their overall capabilities, this writer was not very convinced of their claims.

B. All Union Institute for Hydrolysis, Leningrad, USSR
(U.S. Team: Leise, Tsao, Field, Arminger, Wang)

On Thursday, September 25, 1975, a visit was made to the All Union Institute for Hydrolysis in Leningrad. At this Institute we met with:

Dr. Lepidiv (Director of Institute)
Dr. Golavin (Director of Production)
Dr. Shimoshina (Growth of Yeast on Hydrolysate)
Dr. Korikov (Hydrolysis Equipment)
Dr. Millavanov (Cellulose Hydrolysis, Furfural Production)

The visit to this Institute consisted entirely of a session with questions and answers. We were not shown the laboratory and equipment since they stated that the entire laboratory is being physically moved to another locality. There were seven specific questions which were asked by the U.S. delegation. The questions and the responses are briefly summarized.

1. Types of raw material presently used in their hydrolysis plant and products from the hydrolysis plants?

The raw materials consist of corn cobs, woodcutting waste, sunflower shells and cotton seed hulls. The products from these cellulosics were alcohol (ethanol only) and feed yeast. It should be noted that the research on the hydrolysis of cotton seed hulls by microorganisms was confirmed to be carried out in Krasnodar by Dr. Livanoga.

2. Present status of the treatment for solubilization of lignins and their utilization?

It was stated that the utilization of lignins is not fully solved. The Soviets do not have an economical process or product from lignin for large scale exploration. Research is presently being conducted to achieve better utilization, although they did not identify specific avenues. Presently, the lignins are used as fuel but they do not believe this is the most optimal. Some efforts are being directed to obtaining activated carbon as an adsorbent from lignin. Fertilizer from lignin using NH_4OH is also being examined at the research level. Other uses of lignin include those in the metallurgical industry as well as uses in road construction. Their present process does not solubilize lignin as a pretreatment for cellulose hydrolysis. One therefore concludes that the lignin is a byproduct of the cellulose hydrolysis.

3. What type of cellulose hydrolysis plants (processes) are in operation in the USSR?

The raw material, for example sawdust or wood waste, is first mechanically milled to achieve the desired size. This is then placed into the digester (cooker) with 0.5% H_2SO_4. Hydrolysis in acid is accomplished at 10 atmosphere pressure and 200°C. The cellulose is hydrolyzed and the mixture is then filtered to remove the lignin. The hydrolysis is carried out in the equivalent of a "two-stage operation". During the earlier portion of the hydrolysis, five-carbon sugars are obtained and, in the second and latter stage, one obtains six-carbon sugars. Although their present process uses the concept of multiple stages, in reality the process does not contain two discrete stages. Continuous multistage operation is presently under investigation.

4. Is vapor phase hydrolysis of cellulose practiced, and to what extent, in the USSR?

It was not totally clear to this writer whether vapor phase hydrolysis is indeed practiced in the USSR. However, the impression was that vapor phase hydrolysis was employed in Riga in the production of furfural.

5. What methods or techniques are the Soviets using in the milling of cellulose?

The most common milling practice is the mechanical mill. However, a combination of mechanical and chemical pretreatment (H_2SO_4 vapor) has been tested. The cost for this combined treatment is quite high, especially with respect to the material of construction requirement. It was learned that the combined milling process is being examined in Riga (Latvia). Their present process entails the addition of H_2SO_4, and milling using roll mills where hydrolysis of cellulose is achieved. During the milling operation several passages are required. A thick paste results and water is added, followed by heating to 120°C. This mixture is then processed for the isolation of the sugars.

6. Desires of this Institute for the exchange of scientific personnel?

They are interested in scientific personnel exchange but we should handle this through Mr. Shenderey.

7. What is the status in the USSR on the microbiological degradation of cellulose?

The studies on the microbiological degradation of cellulose have just begun in the Soviet Union. Dr. Livanoga is responsible for this research. The interest in this subject is great, but few results have been gathered. Their present approach uses a white rot fungi in solid-state fermentation systems. Their emphasis is on degradation of the lignin fraction of the cellulosics.

C. Institute of Microbiology and Virology, Academy of Sciences, Ukraine, Kiev, USSR
(U.S. Team: Field, Humphrey and Wang)

A visit to the Institute of Microbiology and Virology of the Ukraine Academy of Sciences, Kiev was made on Friday, September 26, 1975. At this Institute we were met by:

Dr. V.G. Kraev (Deputy Director)
Dr. Malenshenko
Dr. Podgorsky
Dr. I.V. Shchelokova (female)

Dr. Kraev presented an overall picture of the Institute, which was established in 1928. The general areas of their research endeavors include:

Physiology and biochemistry of soil microbiology
Nitrogen fixation
Organic substrate transfer by soil microorganisms
Interactions of microorganisms and higher plants
Microbiology of eco-system
Investigation of pathogenic bacteria
Microbiological studies in cancer development
Industrial utilization of microorganisms: bacteria, yeasts and fungi
Biosynthesis of proteins, vitamins and antibiotics
Virology: higher plants, algal viruses, and animal viruses

The discussion that ensued focused mainly on Dr. Malenshenko, whose main interest was in the area of biosynthesis of proteins. It was stated that their research had started about 7 years ago with the main emphasis at the beginning on the use of gaseous hydrocarbons such as methane, ethane, and propane. They isolated an organism (not specified) which utilizes C_1, C_2 and C_3 gaseous hydrocarbons. However, this organism could not utilize derivatives of C_2 or C_3 gases (e.g. acetic acid). The derivatives of methane were utilized. Within the Institute it was stated that a computer (Soviet make) was coupled to a fermentor (Soviet make) which optimizes the transition from batch to continuous operation. This writer is not certain of the exact optimization technique or the role of the computer in such a system. The optimization studies involved the use of O_2, temperature, biomass concentration and pH. Productivity of such a gaseous SCP scheme ranges from 0.5 to 0.6 gm/L-hr. Temperature of growth was 55°C and a substrate yield of 0.15 to 0.20 gm cell/gm substrate was achieved.

Dr. Podgorsky proceeded to discuss their research on the use of methanol for SCP production. The people at this Institute appear to have some cooperation with Dr. Eroshin (Putchino) on the studies with methanol. The studies using methanol started in 1969 and their emphasis is on yeast only! The organism under

study is Candida boidinii, having a growth rate of 0.2 hr^{-1} and cellular yield of about 35%. They have performed some detailed studies on double nutritional limitations (O_2 and CH_3OH) in continuous culture. A 1975 reprint in Russian was given to us by Dr. Podgorsky.

It was very unfortunate that due to limited time we were unable to find out the nature of the research of Dr. I.V. Shchelokova. She apparently is the person conducting the research on cellulose degradation by microorganisms. Lastly, we did not see any laboratory or equipment at this Institute.

D. Gas Institute, Kiev
(U.S. Team: Field, Humphrey and Wang)

Also on Friday, September 26, 1975 a visit was made to the Gas Institute at Kiev. We were met by:

Dr. Konstantin Makhorin
Vice Director
Institute of Gas
Kiev - 113
39 Parkhomensko St.
USSR

The Gas Institute is involved in the microbial synthesis of protein from natural gas. Dr. Makhorin does not appear to have any technical background in microbiology. The only effort in the SCP area is a (pathetic) 1-liter fermentor still under construction for natural gas fermentation. This system can best be described as an assemblage of "junk" which when completed would have absolutely no bearing on the success of any program. It consists of some pieces of old pipes and an agitated fermentor capable of operating at 50 atms. Thus far no experimental studies have been conducted. This writer doubts that any experiment will ever be achieved in this system.

E. Institute of Light Chemical Industry, Moscow, USSR
(U.S. Team: Wang, Field and Arninger)

On Monday, September 30, 1975, a visit was made to the Institute of Light Chemical Industry in Moscow, USSR. We were met by Dr. Nicholyav, who is a chaired Professor in microbiological processes. We were informed that this Institute is an educational institute having approximately 5500 students, of which 350 are post-graduates. A total teaching staff of 450 is at this Institute, of which 40 are professors and doctors. The main emphasis of this Institute is on mechanical design of chemical processes.

The Department of Microbial Processes with which Dr. Nicholyav is affiliated is about 14 years old. They are primarily interested in the design of equipment for microbial technology. They

employ "modern" theory but all of their work is industrial or practical-oriented. Dr. Nicholyav proceeded to illustrate their inputs on the continuous production of vinegar (acetic acid) from ethanol at a pilot-scale level. It was stated that their Institute designed and constructed a five-stage continuous vinegar generator, each stage approximately 10 m^3 in volume. The final output from this process is vinegar having acetic acid concentration ranging from 9 to 12% on a continuous basis. It was stated that this type of vinegar production is practiced throughout Russia, using the developments from their Institute. In the process of their research and development, a great deal of work was performed on aeration and agitation to achieve the desired mass transfer.

From the conversation with Dr. Nicholyav, it is apparent that their main emphasis is on mechanically agitated fermentors. Furthermore, it was also apparent that this Institute is instrumental in the scale-up of fermentors. Dr. Nicholyav does not appear to be enthused with the airlift fermentor for SCP production on hydrocarbons. He did mention that during scale-up of the airlift fermentor, the height of the fermentor is critical, but the diameter of the unit is not so important. For example, the height should be at least 6 meters. It was also Dr. Nicholyav's opinion that the airlief fermentor cannot achieve high rates of oxygen transfer. For example, he stated that the maximum productivity of biomass on hydrocarbons will only be 1.5 to 2.0 gm/L-hr in the airlift type of fermentors. He believes the only way to achieve high oxygen transfer is through mechanical agitation. For example, he quoted typical high power inputs employed in their mechanically agitated fermentor of about 40 kw/m^3 (200 HP/1000 gallons)! This is incredibly high, but Dr. Nicholyav was quite insistent on this matter. Typical O_2 utilization efficiency ranges from 25 to 40%.

Lastly, Dr. Nicholyav also mentioned that other Departments are doing research in the area of separation technology. Time did not allow us to pursue this in more detail.

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TRIP REPORT OF INSTITUTE VISITS IN CONNECTION WITH THE 4TH MEETING OF THE US/USSR JOINT WORKING GROUP ON PRODUCTION OF SUBSTANCES BY MICROBIAL MEANS MOSCOW, SEPTEMBER 22-30, 1975

by A.E. Humphrey

In connection with the 4th Meeting of the US/USSR Joint Working Group on Production of Substances by Microbial Means in the Soviet Union, September 22-30, 1975, the following visits were made:

Monday, September 22 - All Union Institute of Protein Synthesis, Moscow, V.K. Gradova, host

Thursday, September 25- All Union Research Institute for Hydrolysis Leningrad, Dr. L.V. Dmitriyenko, Director

Friday, September 26 - UAS, Institute for Microbiology and Viriology, Kiev, Dr. V.G. Kraev, Deputy Director

Friday, September 26 - UAS. Institute of Gas Technology, Kiev, Dr. K. Makhorn, Vice Director

Saturday, September 27 - Moscow State University, School of Chemistry, Dr. I.V. Berezin, Dean

In addition a number of interesting conversations on SCP production were held with Dr. S. Yenikeyev, Kazan Institute of Chemical Technology, and on Enzyme activity in the USSR with Drs. A.M. Egorov and A.A. Klyosov of the Laboratory of Chemical Enzymology of Moscow State University.

All Union Institute of Protein Synthesis

The whole delegation was taken on a tour of the laboratories including those for:

(1) Microbiology
(2) Physical Chemistry
(3) Analytical Chemistry
(4) Fermentation Pilot Plant

The Microbiological Laboratory is headed by Dr. (Mrs.) V.K. Gradova. The working appears mostly focused on screening and strain improvement for hydrocarbon utilizing yeasts.

The Physical Chemistry laboratory is headed by Dr. A.D. Gololobov. The work is focused mainly on the biochemistry of n-alkane uptake by yeast. The level of this work was most impressive. It appears that Dr. Gololobov has a very good understanding of hydrocarbon uptake mechanisms.

The Analytical Laboratory was well equipped for amino acid, fatty acid, benzephrene, and other important component analysis of hydrocarbon utilizing yeasts. The laboratory does all nitrogen analysis by pyrolysis which has been automated and protein analysis by amino acid analysis.

The pilot plant seemed to be well equipped but not in use. The NBS computer-coupled fermentor has been installed in the pilot plant. However, it is not operable due to non-functional dissolved oxygen control and non-operable computer interface. The equipment has been in place for nearly one year. Service from the DEC and NBS companies have been most unsatisfactory. As a result the Soviets are discouraging persons from buying NBS equipment and have just ordered a similar system from Sweden but based on specifications obtained from reports on the system at the University of Pennsylvania.

All Union Research Institute for Hydrolysis

A two hour question and answer period was held at the Institute with Dr. L.V. Dmitriyenko, the new director, and Mr. Vadim V. Golovin, General Director of Gydrolizprom (All Union Hydrolysis Corporation). We were told that there were roughly 50 hydrolysis plants in the USSR, mostly operating

on coniferous wood wastes, although some plants operate on agricultural wastes such as corn cobs, sunflower seeds, and cotton seed balls. A typical output for these plants would be 6000T/year of yeast protein. However, alcohol, CO_2, and furfural are also produced in some of the plants which use an acid hydrolysis process. A typical fermentor is the order of 1,350 kiloliters in size. They usually use a 1% cellulose charge. Typical hydrolysis conditions involve a 2 stage system (in place) using 0.5% H_2SO_4 at 10 atm pressure and 200°C. They begin with 150-180°C, flushing mainly the permicellulose, and then in the second stage increase the temperature to 200°C, flushing mainly the hexoses.

They have developed a VN11G mill for improving sugar recovery from the cellulose. This unit is at the Riga Institute. It consists of high pressure rolls (several micron size range gap) that handles either cellulose moistened with H_2SO_4 or exposed to SO_3 during milling. This produces a hard to handle paste. Mr. Golovin is opposed to using the process because he feels it is not economical and that it consumes too much electricity.

The Institute is not studying enzyme hydrolysis but is interested in comparing enzyme hydrolysis with acid hydrolysis. They have been looking at the growth of yeast on pig manure but with little success. Apparently the high salt concentration inhibits the yeast growth.

Institute for Microbiology and Virology

At the Institute we had two hours of discussions with Mr. S. Malashenko on the biosynthesis of protein from gas by a pure bacterial strain of a Pseudomonas, with Dr. V.S. Podgorsky on the biosynthesis of protein from methanol utilizing yeast and with Dr.I.V. Shelokova on the biosynthesis of protein from agriculture wastes. The work on gas seemed to be divided between organisms that utilized pure methane and those utilizing natural gas (containing methane, ethane, and propane). Mr. Malashenko claimed he had developed a pure strain of bacteria that could utilize natural gas. This organism is being turned over to the Institute of Gas Technology to test in a pilot plant. In addition, Mr. Malashenko said he was working both with mesophilic and thermophilic bacteria. He said he had developed a process that was optimized by a computer-coupled fermentation system similar to that in the Institute for the Physiology and Biochemistry of Microorganisms at Pushino na Oka. He claimed it had dissolved oxygen,

pH, temperature, and biomass control. This statement probably applies to what he would like to do as the pilot plant at the Institute of Gas Technology only had temperature and pressure control and was not coupled to a computer.

The work on methanol by Dr. V.S. Podgrosky appeared to be very good. He has developed a yeast-methanol SCP process having a $\mu_{max} = 0.2\ hr^{-1}$ and $Y_x/CH_3OH = 0.35$ gm/gm. Further he has optimized the rate of O_2 and substrate addition characteristics. He claims he has submitted a proposal to Mr. Balyaev, Head of the Office for the Microbial Industry, to consider developing an industrial process for processing SCP from methanol.

Very little information was learned from Dr. (Mrs.) I.V. Shelokova about the biosynthesis of protein from agricultural wastes. We gathered that she is cooperating with the Hydrolysis Institute and the Plant in Krasnador. We understand that this plant is an acid hydrolysis plant operating on corn cobs, sunflower seed hulls and stalks (?) and cotton seed hulls. Apparently, Dr. Shelokova is selecting optimal media conditions for yeast culture on these waste hydrolysates.

Institute of Gas Technology

The visit at this Institute was limited to an inspection of a 10 liter pilot plant (28-34°C, 10 atms.) for producing SCP from natural gas and methane. The construction and unit was extremely primitive. It appeared to be constructed from cast off parts and not to have operated. There was no apparent facilities for processing the product beyond the culture stage. The engineers at the Institute were very eager to talk to us and mostly asked questions about dissolved oxygen, CO_2, and pH measurement at 6-10 atms. Our evaluation of the facility was one of extreme pessimism over whether the unit could operate sterilely at 10 atmospheres.

Moscow State University

A visit was made to Dr. I.V. Berezin's Laboratory for Chemical Enzymology. He presently has 125 persons (students, technicians, and researchers) working on enzyme problems. These include light sensitive systems, faint sound sensitive systems, co-factor regeneration and utilization, and cellulose degradation. By 1978 he will have a total building devoted to enzyme chemistry and hopes to have 350 persons working for him. The work on light and accoustically sensitive enzyme systems is extremely impressive. A plant is being constructed to print

patterns by an enzyme technique. We saw samples of the work and were greatly impressed. At present a major thrust is being made to study cellulose degradation by enzymes. Dr. Berezin hopes to produce commercial cellulose enzymes in a few years. This interest was confirmed by the fact that over forty faculty and research technicians (no students) attended my lecture on:

"Degradation of Cellulosic Materials by Enzymes and Microorganisms"

USSR Enzyme Activities

The Moscow State University Chemistry School has decided that major industrial opportunities exist in enzyme chemistry. They are building a large laboratory facility devoted solely to this activity that will house 350 workers. This one institute is spending nearly 1.5×10^6 rubles/year on enzyme technology (applications). Examples of the scope of USSR technology lies in their achievements in application of sound and light sensitive enzyme systems, effort on cellulose enzyme systems, and the development of an immobilized acylase system for synthesis of ampicillin. Since the total US effort is less than that at Moscow University, the US lead in enzyme technology may soon be overcome by the USSR.

SCP - n-alkane Plants

Mr. Shenderey has stated that the Gorky SCP plant had a productivity of 20 gm/liter-hour. Conversations with Soviet Scientists who claim to have been involved indirectly in the design of the plant indicate that this was the hoped for productivity. In fact it is much less than this, probably nearer ¼ of the value stated by Mr. Shenderey. The Gorky plant is a multi-stage operation with mechanical agitations and over feeding. Unfortunately, the system was not optimally designed so that the planned productivities were not achieved. The system is apparently being modified. High O_2 transfer rates are claimed to be achieved by a facilitated transport system. An agent such as fatty acid, for example, is used to facilitate the O_2 transfer. Mr. Shenderey claims that the new facility being built at Kiri (Kirishi?) has 3-4 times the capacity of the Gorky plant and embodies the latest technology and process developments. In the next 6-12 months he hopes to open this plant to foreign industrialists in order to licence the process. He already has inquiries from Germany, France, England, Italy, Holland, Norway, Sweden, and Japan, but none from the U.S.

UNIVERSITY OF PENNSYLVANIA

The subtask activity at the University of Pennsylvania is concerned with the production of Single Cell Protein from cellulosic wastes (Bagasse, Hay, etc.) using a Thermoactinomyces sp. A model that simulates the microbial attack of cellulose has been devised and is now being improved to more nearly represent real situations. To accomplish this task, a computer coupled fermentation system has been developed. The system is being used to test and evaluate techniques for biomass estimation by fluorometry and oxygen uptake. It is also being used to study process kinetics with on-line analysis of sugars by immobilized enzymes and high pressure liquid chromotography. At this time, the system is fully operable and is being used to generate kinetic data for modeling the cellulose fermentation. Eventually, the system will be used for control and optimization of this cellulose utilization process.

KANSAS STATE UNIVERSITY

The work at Kansas State University is coordinated by Dr. Larry Erickson in cooperation with Dr. L.T. Fan. Two subtasks have been organized. One deals with modelling the kinetic characteristics of the yeast Candida lipolytica growing on dispersed systems of hexadecane. Variables which are being measured to characterize the system dynamics with respect to dispersion and mass transfer include dissolved oxygen concentration, n-hexadecane concentration, fatty acid concentration, surface and interfacial tension and oil drop size. The other study deals with the application of the GMDH (Group Method of Data Handling) to on-line optimization of the hydrocrabon fermentation.

Preliminary results indicate that a satisfactory kinetic model will be obtained to predict cell growth in various hydrocarbon systems. Also, the GMDH method appears to be useful to on-line optimization of hydrocarbon fermentations.

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MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Task 2 activity at the Massachusetts Institute of Technology is coordinated by Dr. Charles Cooney. Dr. Daniel I-C Wang is also associated with the subtask. One aspect of the task is the measurement of heat of fermentation and its correlation with cell mass production using a computer-coupled fermentation system. A dynamic calorimeter technique has been developed. This is being coupled with a CO_2 evolution measurement and a viscometry measurement in order to estimate cell biomass and cell activity rapidly and continuously.

Additionally, the group is looking at the modelling of mixed culture systems. Specifically, the group has studied the kinetics of 2 yeasts growing on 2 carbohydrates. This work is being expanded to study a mixture of yeast growing on impure methanol (mixed alcohol) substrate.

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Trip Report: USSR--September 21-30, 1975

Meeting of US/USSR Working Group on the

Production of Substances by Microbiological Means

By Edmund Field

Project #1, Single Cell Protein

The first item of business was to reconcile the revisions to the Working Program by the US and USSR teams. Project #1, whose title was revised by mutual agreement to "Single Cell Protein", was represented on the US side by Dr. Daniel I. C. Wang and Dr. Edmund Field. The USSR coordinator, Alfred Gregorian, was ill, and we worked with Dr. N. B. Gradova of the Institute of Protein Synthesis, State Office of Microbial Industry, Moscow. Assisting her was Dr. V. Eroshin from the Institute of Biochemistry and Physiology of Microorganisms, Academy of Science of the USSR, Puschino.

The changes from the Working Program prepared in 1974 proceeded smoothly in an atmosphere of friendly cooperation and mutual respect. Many changes were for clarification of language and reconciling translations and need no comment.

Target dates were changed to reflect the current timing situation. Amplification of the Working Program was suggested by the USSR team and were agreed to by us: (1) Development of physiological, cytological and biochemical tests for selection of bacteria, yeasts and fungi and (2) Development of improved methods for storing microorganisms in a way which preserves their valuable characteristics.

We also made tentative agreements for exchange of scientists in 1976.

The abrupt cancellation of the US/USSR scientific conference scheduled for MIT in October was dismissed by Gradova as connected with Dr. Gregorian's illness. Before we left Moscow we had a fairly firm agreement to reschedule the conference at MIT for December 16-18, 1975. We were provided with a list of ten papers with titles and authors, and a promise of abstracts to be sent to Dr. Wang shortly.

Coordinator's Reports

The coordinators for both sides were asked to report on their progress and plans for each project area. I will summarize only Project #1, presented by Dr. Wang and Dr. Gradova.

Dr. Gradova reported that the USSR is making progress on all the aspects listed in our Working Program. They have

isolated selected strains for methanol, ethanol, carbohydrates and cellulose as well as for n-paraffins. No work on methane is underway (we found work on methane in progress in Kiev). They have induced mutants with protein content above 65%, with high methionine content, and high yields on substrate (apparently for hydrocarbons primarily). Work is in progress on the mechanism of attack on substrate and on the biochemistry of the organism. With methanol they have achieved yields of 40% with yeast and 45-50% with bacteria. They are designing mathematical models to help compare production costs from laboratory data, and are making techno-economic evaluations for biomass produced from hydrocarbons, methanol, ethanol and molasses. They also are working on biomass recovery, and on isolation and fractionation of protein therefrom. Scale-up problems and evaluation of functional and biological value of the biomass are also underway. Most of this work will be reported in our MIT conference in December.

Dr. Wang reported that much US work in this area is not specifically sponsored by the exchange program. He reviewed the seven sponsored projects, and it was obviously not a coordinated effort in contrast to the USSR. Shendery pointed out the weakness of the US effort in this area and suggested that the USSR ought to cut back. He believes that the US will

need this program in 10-20 years, and suggests that we review our priorities.

Laboratory Visits

We were escorted through the laboratories at the Institute for Protein Synthesis in Moscow. It was obvious that they have a large and well coordinated staff effort. Much equipment was out of date, and most instruments were imported. We saw more abacuses than calculators. The only significant piece of information I picked up was their claim that the carcinogenic benzopyrenes in yeast grown on purified n-paraffins comes from polluted air and is higher in winter than summer. Thus plant location and air purity could be critical for producing an acceptable food grade biomass.

We visited briefly the Cellulose Institute in Leningrad but saw none of their laboratories. They are primarily interested in utilizing wood and wood by-products, but are also working on agricultural waste. Utilization of lignin is a major unsolved problem. I believe they learned more from Drs. Humphrey and Tsao than we learned from them. Paralleling US work on utilization and recycling of cattle waste from feed lots, the USSR is concerned with waste from pigs. They have been unable to grow yeast on pig waste.

The following day we (Field, Humphrey, Wang) made a hectic trip to Kiev (the full story is reserved for cocktail parties). At the Institute for Microbiology and Virology, Academy of Science, Ukrainian SSR, we met with Dr. Kraev and his staff. Topics of interest to us included soil microbiology, microbiology of cancer, production of bacteria on methane, ethane, and propane, and on methanol. The work on gases became focused on methane. They tried to use computer programs to translate from batch to continuous operation and to design for scale-up. They started growing yeast on methanol in 1969 and have isolated several strains. They achieved an 0.2 $hour^{-1}$ growth rate with a 35% yield on methanol. They published a paper reporting that growth is limited by both oxygen and methanol concentration. Work at this institute is largely theoretical; they claim close contact with the group at Moscow.

In Kiev we also visited the Gas Institute where they are apparently trying to translate the theoretical work on methane to a pilot plant. They plan to start at ten atmospheric pressure, once through, with plans for gas recycle later. The equipment is very crude and no useful results are likely to arise.

On our return to Moscow we visited the Institute of Machine Design. This is really an educational institution with

primary emphasis on chemical engineering. Dr. Nicolaev is head of the microbial engineering department and acting head of the Institute. He told us of his achievements in producing food vinegar in a five stage fermentation process from ethanol. For hydrocarbon fermentation, vigorous agitation is required, and to avoid mechanical seal problems, he recommends an air lift. Although the Institute is involved in design of the commercial yeast plants, our visit was not very fruitful.

Closing Discussion

Our failure to see any of the USSR commercial plants was tied by Shendery to our inability to show them what they want to see in the US. Reading between the lines, I concluded that they are having delivery and performance problems on equipment components and they have nothing to show. Shendery stated that their hydrocarbon fermentation process is simpler and better than the competition, and he will be prepared to license their process in six to twelve months.

TRIP REPORT OF INSTITUTE VISITS IN CONNECTION WITH PROJECT 2, TASK 2 OF THE US/USSR JOINT WORKING GROUP ON THE PRODUCTION OF SUBSTANCES BY MICROBIAL MEANS SEPTEMBER 21-OCTOBER 12, 1975

BY

W.B. ARMIGER

In connection with the Project 2, Task 2 of the US/USSR Joint working Group on Production of Substances by Microbial Means the following visits were made by William B. Armiger of the University of Pennsylvania:

September 22 - 24	Attended the 4th Meeting of the US/USSR Joint Working Group at the All Union Institute Protein Synthesis, Moscow.
September 25 - 26	All Union Research Institute for Hydrolysis, Leningrad, Dr. L.V. Dmitriyenko, Director.
September 27	Moscow State University, School of Chemistry, Dr. I.V. Berezin, Dean.
September 28	Institute of Chemical Machine Design, Dr. P.I. Nikolyov.
September 30 - October 1	Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Puschino, Dr. V.K. Eroshin.
October 2 - 3	All Union Institute of Protein Synthesis, Moscow, Dr. N. Postnikov.
October 4 - 11	Kazan Institute of Chemical Technology, Kazan, Dr. S. Yenikeyev, Chairman Department of Chemical Cybernetics.

On the first day, a two hour question and answer period was held at the Institute with Dr. L.V. Dmitriyenko, a new director, and Mr. V.V. Golovin, General Director of Gydrolizprom (All Union Hydrolysis Corporation). At this meeting we were told that the Institute supports 12 hydrolysis plants throughout the Soviet Union. These plants are used for producing alcohol, carbon dioxide, and furfurol from carbohydrate and carbon containing raw materials such as coniferous wood wastes, low quality wood, and agricultural wastes such as corn cobs, sunflower seed hulls, and cotton seed hulls. The cellulose hydrolysis is carried out with H_2SO_4 at 200°C and 10 atm. (The process involves both chemical treatment with H_2SO_4 and mechanical milling in several stages). At this time, the lignin is not being fully utilized. There is one plant, however, that is using it as a low quality fuel, and in another instance it is being used to produce charcoal. The Institute has studied hydrolysis in the presence of gaseous H_2SO_4. This technique results in improved digestability, but here are equipment limitations. The process is not very efficient and results in a high consumption of electricity so that at the present time it is not being applied to industrial processes.

On the second day of my visit I gave a seminar on "The Production of Single Cell Protein from Cellulosic Wastes using Thermoactinomyces." The seminar was followed by a question and answer period during which there was much discussion about the enzymatic hydrolysis of cellulose. The Institute for Hydrolysis is not studying the enzymatic hydrolysis of cellulose; however, they are very interested in comparing enzymatic hydrolysis with acid hydrolysis.

After the seminar we discussed in more detail the work being supported by the Institute for Hydrolysis. One project discussed involved with the production of single cell protein in the form of yeast from the hydrolysates of wood. There are approximately 50 single cell protein plants around the Soviet Union that are used for the continuous production of yeast. The raw materials for the fermentation consist of wood chips and saw dust. The first step is the hydrolysis which takes place in an 80 meter vessel at 200°C in .5% H_2SO_4. This is a batch process. After neutralization and sedimentation the product of the hydrolysis then goes to the fermentor. The industrial scale fermentors are approximately 1300 cubic meters and operate with a working volume of about 800 cubic meters. The feed stream to the fermentors consists of a 1% solution of sugars at a flow rate of 50 cubic meters per hour. The output from the fermentors is 50 cubic meters per hour with .1% sugar reducing substances in the product stream. The fermentors are operated under non-asceptic conditions. The research at the Institute supporting this process is concerned with the selection of microorganisms, the stability of a continuous fermentation, and the optimization of the process. Following the fermentation the product stream then goes through a concentration phase and drying. The product is used as feed yeast for both chickens and pigs. Another project being investigated

at the Institute for Hydrolysis is the production of fungi directly on saw dust for use as a feed. At this time they are working with [illegible] organisms and investigating the growth rates of these organisms. A third project discussed involved the utilization of pig manure from feed lots. The feed lot wastes consists of about 40% cellulose. This material first goes through the acid hydrolysis process to produce a sugar solution. The sugar solution is then used in a fermentation to produce yeast. However, the growth of the microorganisms is inhibited by impurities in the feed lot materials. This work is being done in laboratory scale fermentors of 3-10 liters.

Moscow State University

A visit was made to Dr. I.V. Berezin's laboratory for Chemical Enzymology. The work in this laboratory deals with light and sound sensitive enzyme systems, co-factor regeneration and utilization, cellulose degradation and energy conversion with biochemical fuel cells. The work with light sensitive enzyme systems was very impressive. We were shown a print of a pattern produced by an enzyme technique. The biochemical fuel cell working with hydrogenases was reported to be capable of producing an output of about 100 watts per liter.

Institute of Chemical Machine Design

We visited the office of Dr. P.I. Nikolyov of the Institute of Chemical Machine Design Moscow. This is an educational institute for training engineers and mechanics for the chemical industry. The Institute has about 5000 students, 350 post-graduate students, a teaching staff of about 450, 40 of which are professors or Ph.Ds. Dr. Nikolyov works in the area of design of microbial equipment and processes. He is a chemical engineer by training and is involved in transferring this experience into microbiological technology. Most of the time was spent talking about a process for the production of acetic acid that was developed at this Institute. This process is used at the Institute as a model for investigating other microbial processes & for research on aeration and mass transfer. Acetic acid production in a batch process is limited by product inhibition at a concentration of about 9%. For this reason they have designed a continuous process that utilizes a five stage fermentor. This configuration enables them to achieve a 12% concentration of acetic acid in the final product stream. There is one commercial plant utilizing five 10 cubic meter fermentors which produces about 500 kilograms per hour of 9% acetic acid. While there we discussed other topics such as oxygen mass transfer in fermentors. Dr. Nikolyov agreed with us that the figure of 20 kilograms per cubic meter per hour of oxygen transferred in a hydrocarbon single cell protein fermentation was not possible. This is in direct contradiction to Dr. Shenderey's claim and is further evidence that Dr. Shenderey may be stating goals rather than accomplishments.

At the Institute I spoke briefly on "Mathematical Modeling the Cellulose Fermentation." I was also taken on a tour of the laboratories, which included:

(1) The Laboratory for Microbial Transformation of Organic Compounds
(2) The Department of Energetic Metabolism
(3) The Laboratory for the Cultivation of Microorganisms
(4) The Laboratory of Morphology and Biophysics of Microorganisms
(5) The Laboratory of Physiology and Growth of Microorganisms
(6) The Laboratory of Microbial Technology
(7) The Calculation Techniques Laboratory

The Laboratory for Microbial Transformation of Organic Compounds is headed by Dr. L.A. Golovleva. Research in this laboratory is concerned with the microbial transformation of steroids and the degradation of herbicides. There are two new laboratories that are being developed, one dealing with the enzymatic modification of nuclic acids and another on molecular biology. They are also doing some work in this laboratory with immobilized enzymes.

The Department of Energetic Metabolism, headed by Dr. A.B. Losinov is concerned with oxidation metabolism. In particular they are studying changes in metabolic pathways related to substrate and oxygen concentration. They are interested glucose and n-alkane fermentations as well as intermediates in the degradation of n-alkanes, such as alcohol. The results of their work show that the critical oxygen tension is different for growth and respiration using either glucose or n-alkanes. An oxygen concentration of greater than 30% is need for growth, whereas 4-6% if sufficient for respiration. They are also working on comparing the TCA cycle for both glucose and n-alkane fermentations.

The Laboratory for the Cultivation of Microorganisms is headed by Dr. A.N. Shkidchenko. The pilot plant is used for the large scale production of biological materials and for process development. The pilot plant which is still under construction will contain, four 40 liter fermentors, ten 100 liter fermentors, and two 3 cubic meter fermentors. All fermentors will be capable of continuous operation. They will have pH control, temperature control, and sensors for minitoring dissolved oxygen. I was told that some of these fermentors will be interfaced to a computer, but I did not see sophisticated equipment for interfacing so I have doubts about how effective they will be in utilizing computer control in their pilot plant.

The Laboratory on the Morphology and Biophysics of Microorganisms is headed by Dr. B.A. Fichter. It is primarily concerned with the physical destruction of microorganisms. The laboratory uses a variety of techniques including a Hughes press, ultrasonics, extrusion through a French press, and an Ultrasonic Centrifuge.

The Laboratory of Microbial Technology is headed by Dr. V.K. Eroshin. We had a long discussion in this laboratory with Dr. I.G. Minekevich on material and energetic balances related by oxygen consumption. The first step is to write a generalized equation for the elemental balance of microbial growth on a carbon substrate. This is used to calculate the yield of dried biomass based on the consumed oxygen. The yield on oxygen is found to be dependent upon two factors, (1) the carbon yield and (2) the reduced degree of the substrate. The dependence of the yield on these two quantities is then related to a single factor that they call the energetic yield of growth. Using this concept they are able to calculate the restrictions on the range of workable carbon yields during growth on various substrates. They have also shown that the metabolic heat generation of the fermentor is proportional to oxygen consumption and averages about 3.38 kilocalories per gram of oxygen, regardless of the substrate and microorganisms used. I was given several recent publications dealing with these concepts.

The Laboratory of Calculation Techniques is headed by Dr. I.S. Utkin. We had several discussions on the applications of computers for optimizing fermentation processes. We also discussed a technique they are working on for calculating μ_{max} and K_S by measuring biomass during the transition between steady states of a continuous fermentation.

My overall impression of the Institute of Biochemistry and Physiology of Microorganisms in Puschino is very good. I found the laboratories to be well equipped with both Eastern European and Western instrumentation. I also found the laboratories to be well staffed and the people more than willing to talk about the work that they are doing and to answer questions. I think that the Laboratory at Puschino is a worthwhile place for setting up further avenues for cooperation.

The All Union Institute for Protein Synthesis

At the All Union Institute for Protein Synthesis in Moscow I was taken on a tour of the following laboratories:

(1) Biochemistry
(2) Analytical Chemistry
(3) Automation
(4) Fermentation Pilot Plant

The Biochemistry Laboratory is headed by Dr. A.D. Gololobov. Their primary interest is in the assimilation of hydrocarbons by microorganisms. Specifically, they are interested in microbial adaptation to hydrocarbon metabolism, with two purposes; (1) the control of the process and (2) the metabolism of paraffins after enzyme induction.

The Analytical Chemistry Laboratory is headed by Dr. R. M. Fedorovich. It is primarily an analytical support laboratory for the hydrocarbon fermentation. The laboratory is very well equipped, mostly with Western European and US instrumentation. They measure specific ions by atomic adsorption spectra. They have a number of automated systems for measuring amino acid composition, nitrogen composition, and hydrocarbon composition. I tried to determine, specifically why they were interested in measuring certain ions; however, I was told that this was merely a support laboratory which meant that they were doing primarily analysis for other laboratories. I tried to pursue, specifically, who was interested in the ion work, but it was very difficult to get a name. I could not tell whether they were not being cooperative or whether Dr. Fedorovich really did not know some of the details of the work that was going on.

The Laboratory on Automation, was headed by Dr. I.M. Chirkov. This laboratory is primarily concerned with the development of instrumentation for application to industrial processes. This function will be taken over by the Institute of Biotechnology which were told was being built. I should note that I was unable to see any of the laboratories that are presently used by the Institute. I was told that this laboratory developed an instrument for determining biomass by measuring cytochrome C concentration. They claim that this instrument is now being used in several hydrocarbon fermentation plants, such as the one at Gorky, Kirishi, and Ufa.. However, they were very reluctant to talk specifically about the industrial application. This laboratory has also developed a dissolved oxygen sensor using a silver cathode and a lead anode. They claim that the probe does not need an amplifier. They feel that this makes it more suitable for industrial conditions. The probe contains a teflon membrane and is not sterilizable. However, they say that on the industrial scale they operate with non-asceptic conditions. The probe seemed to be rather crude; I do not understand why they have spent time developing this particular probe when others, equally as good and perhaps better, are commercially available. I was told that in the future they would like to work on developing instrumentation for heat evolution, and for measuring parameters that will characterize the oxidization and respiration character of microorganisms. In this laboratory, they claim to have a computer coupled fermentation. However, I later found that the computer work is not really interfaced directly to the fermentor but that the input parameters and data are fed manually to it during or after the fermentation. They feed into the computer information on trace element concentration,

protein concentration, product gas analysis, biomass concentration, and hydrocarbon concentration. From this information they calculate stoichiometric coeficients. They would like to include not only programmed methods of control but adapative methods as well. I found Dr. Chirkov to be very defensive in answering many questions and was left with the impression that it would be very difficult to try to cooperate with his laboratory in any meaningful manner. I am doubtful as to whether or not they are really doing as much work as claimed. I asked to see the results of some of their computer programs but was not allowed to do so. My feelings are that I was not shown this information because it probably did not really exist.

The final laboratory that I visited was the fermentation pilot plant which consisted basically of the New Brunswick Scientific computer coupled fermentation system. With this system they have done several studies on oxygen mass transfer, but that is about the extent of the work. At the present time, the total system is still not in complete operation. I was told that people, both from Digital Equipment Corporation and New Brunswick Scientific, were going to return shortly to try to get the system on-line.

My overall impression of the All Union Institute of Protein Synthesis was that it would not be the best place to try and set up formal avenues of cooperation. Either they do not have any information that is worth exchanging or Dr. Shenderey is too tightly controlling what they are allowed to say.

On the second day of my visit to the All Union Institute of Protein Synthesis I presented a seminar on "The Computer Coupled Fermentation System at the University of Pennsylvania". After the seminar, there was a lot of discussion and interest on the work that we were doing by measuring culture fluorescence. Later on in the day, I had discussions with Drs. Babkin, Komorov and concerning the papers that they had given in August at the University of Pennsylvania. I asked if I could see the instrumentation and interfacing with which they were working. However, I was told that they were at the Institute of Biotechnology which was still under construction and hence could not be seen. These discussions turned out to be no more useful than the papers that were presented at the University of Pennsylvania. It is still not clear to me why they seem to be reinventing a number of instruments that are already commerically available.

Kazan Institute of Chemical Technology

At the Kazan Institute of Chemical Technology I visited Dr. S. Yenikeyev who is the Chairman of the Department of Chemical Cybernetics. While I was there I gave two seminars (1) on Modeling the Cellulose Fermentation and (2) on the Development of the Computer-Coupled Fermentation System at the University of Pennsylvania. I also worked with Dr. Rustem Valeyev, Dr.

Sergey Mukhatchev, and Dr. Byacheslav Kropatchev on the model identification problem related to the cellulose fermentation. We discussed and worked on several techniques for identifying parameters and optimizing the process. Several computer programs were being written and one was actually being tested while I was there. However, due to the speed of their computer systems we were not able to obtain final results. However, I was able to return with source listings of the programs they were using so that they could be modified for use on our system at the University of Pennsylvania. I found the people at the Institute to be very cooperative and very interested in setting up avenues of cooperation between their department at the University of Pennsylvania. During the week Dr. Yenikeyev and I had numerous discussions on computer control and on-line optimization of fermentation processes. We also discussed specific ways of coordinating our work which I indicated I would continue upon returning to the United States.

In conclusion, I found the trip to be very useful. I was able to spend enough time at the various institutions to become familiar with the work in process. Since I do not speak Russian, trying to work in the Soviet Union on a long term basis would be very difficult and not very rewarding. Therefore, I think short visits to exchange ideas followed by implementation in each side's home laboratory is a more efficient method for accomplishing our common goals.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTH CENTRAL REGION
Biological Control of Insects Research
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February 6, 1976

Dr. Alioshina, Director
All-Union Res. Institute of Microbial Methods
for Plant Production & Bacterial Preparations
Ministry of Microbial Industry
Lesteva 18
Moscow, USSR

Dear Dr. Alioshina:

The following is a synopsis of our discussion in Chicago concerning implementation of the working program "Microbiological Control of Pests in Agriculture." By mutual agreement, USSR and USA scientists will concentrate their efforts in 4 subject areas. These subproject areas are:

I - Production of Entomopathogenic Fungi

II - Feasibility of In Vitro Production of Invertebrate Viruses

III - Genetics; Phage Resistance; Isolation, Selection, and Development of More Virulent Strains of Bacillus and Beauveria

IV - Standardization Techniques for Assaying Insecticidal Activity of Entomopathogens

Each subproject area will be co-chaired by a USSR and a USA scientist whose responsibility will be to develop specific objectives and coordinate the subproject program under their area of responsibility. Each subproject area, in addition to the chairman, will also be composed of several subproject members.

Attached is a tentative listing of the USA subproject chairmen and subproject members and their areas of specialization. As agreed at our meeting, a similar listing will be developed for USSR scientists. I have left flexible the spacings for USSR scientists so that you might better visualize what the program might entail. You may list as many USSR scientists as you feel are necessary to support the work in each subproject area.

As discussed, the USSR will provide specialists in production of Beauveria, Entomophthora, and Aschersonia for SUBPROJECT I: Production of Entomopathogenic Fungi. Studies under SUBPROJECT III will require specialists working with Bacillus thuringiensis and Beauveria bassiana. SUBPROJECT IV on Standardization will primarily concentrate on Bacillus, Beauveria, and one to three viruses. Our cooperative studies in SUBPROJECT II will focus on in vitro production of the gypsy moth NPV.

The general comments concerning the possible areas of cooperation and expected results previously established are essentially unchanged; namely, to exchange information and personnel and to organize reciprocal symposium-work conferences. These are also provided in the attached document.

An initial symposium-organizational work conference will be developed as soon as specific scientists are assigned to each subproject area. As we discussed in Chicago, the tentative program for the symposium-work conference could follow this general outline:

(1) PRESENTATION PHASE - Each subproject scientist will review past developments and the current status of research in the USSR or USA.

   (a) Anticipated duration: 2½-3 days
   (b) Anticipated number of speakers: 25-30
   (c) Anticipated speaker-time: 30 minutes
   (d) Open discussion period following each presentation: 10-15 minutes

(2) WORKING CONFERENCE PHASE - USSR and USA scientists from each subproject area will separate into their respective groups.

   (a) Anticipated duration: 1 day
   (b) Research objectives will be established.
   (c) A program to implement objectives will be developed.
   (d) A document on objectives and programs will be prepared.

(3) PLENARY DISCUSSION PHASE -

   (a) Anticipated duration: ½ day
   (b) Documented program from each subproject area will be presented to the entire group for review and discussion.
   (c) A consolidated research program for the entire project will be assembled.

If I misinterpreted any of our discussions in Chicago or if there are any areas not clear, we can handle these through correspondence.

It certainly was a pleasure to personally meet you as well as Dr. Skladnev, Dr. Kurenkov, Dr. Zhdanov, Dr. Afrikyan, and, of course, Dr. Smetnik. Hopefully, the balance of your trip to California, Texas, and Maryland was as technically rewarding and personally enjoyable as were our meetings in Chicago.

Sincerely,

Carlo M. Ignoffo
Laboratory Director

Enclosure

cc: Acker
Leise
Sheldon

Trip report to Sept., 1975.

Soviet Research Activities in Enzyme Technology

by George T. Tsao

I am most impressed and alarmed by the strong push by the Soviet Government on Enzyme Technology research. We started our NSF Enzyme Technology program ahead of the Soviets. After 3 years or so of concerted effort involving some 30 research groups at various universities and research centers, NSF Enzyme Technology Program now ceases to exist formally after the recent re-organization. Most of the 30 some groups are now either being disbanded or trying hard to re-adjust their research direction. Even with the most optimistic estimate, three years effort can at best open the door but cannot really lead to true fruition. Since our results are all in the open literature, the Soviets can benefit from our past labor. With their new government, 5-year, funding support program, we can expect that the Soviets will soon bring a number of enzyme processes into industrial production. The following is a list of several such related activities.

(1) Researchers at Moscow State University on light induced enzyme reactions have successfully developed a new photographic technique. Soviets are building a factory for industrial production of photographic pictures based upon enzymatic reactions. Samples of black-and-white pictures were shown to American visitors who were told that research is now under way for developing colored pictures using enzymatic reactions.

(2) Soviets have built a pilot plant for production of glucose from starch using immobilized enzymes. They are anxious to buy American know-how for conversion of glucose to fructose.